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State-space likelihoods for nonlinear fisheries time-series

Perry de Valpine and Ray Hilborn

Abstract: State-space models are commonly used to incorporate process and observation errors in analysis of fisheries time series. A gap in analysis methods has been the lack of classical likelihood methods for nonlinear state-space models. We evaluate a method that uses weighted kernel density estimates of Bayesian posterior samples to estimate likelihoods (Monte Carlo Kernel Likelihoods, MCKL). Classical likelihoods require integration over the state-space, and we compare MCKL to the widely used errors-in-variables (EV) method, which estimates states jointly with parameters by maximizing a nonintegrated likelihood. For a simulated, linear, autoregressive model and a Schaefer model fit to cape hake (*Merluccius capensis* × *M. paradoxus*) data, classical likelihoods outperform EV likelihoods, which give asymptotically biased parameter estimates and inaccurate confidence regions. Our results on the importance of integrated state-space likelihoods also support the value of Bayesian analysis with Monte Carlo posterior integration. Both approaches provide valuable insights and can be used complementarily. Previously, Bayesian analysis was the only option for incorporating process and observation errors with complex nonlinear models. The MCKL method provides a classical approach for such models, so that choice of analysis approach need not depend on model complexity.

Résumé : Les modèles d'espace d'états servent couramment à incorporer les erreurs de processus et d'observation dans l'analyse des séries chronologiques en pêcheries. L'absence de méthodologie classique de vraisemblance pour les modèles d'espace d'états non linéaires est une carence dans ces méthodes analytiques. Nous évaluons une méthode qui utilise des estimations de la densité pondérée des noyaux des échantillons bayésiens a posteriori pour estimer les vraisemblances (MCKL, vraisemblances kernel de Monte Carlo). Les vraisemblances classiques requièrent une intégration dans l'espace des états ; nous comparons donc la méthode MCKL à la méthode très utilisée des erreurs sur les variables (EV) qui estime les états conjointement avec les paramètres en maximisant une vraisemblance non intégrée. Dans l'ajustement d'un modèle d'autorégression linéaire simulé et d'un modèle de Schaefer à des données sur des merlus de cap (*Merluccius capensis* × *M. paradoxus*), les vraisemblances classiques fonctionnent mieux que les vraisemblances EV qui fournissent à l'asymptote des estimations distordues des paramètres et des régions de confiance erronées. Nos résultats sur l'importance des vraisemblances intégrées d'espace d'états appuient aussi la valeur de l'analyse bayésienne avec une intégration Monte Carlo a posteriori. Les deux méthodologies fournissent des perspectives précieuses et peuvent être utilisées de façon complémentaire. Antérieurement, l'analyse bayésienne était la seule manière d'incorporer les erreurs de processus et d'observation dans des modèles non linéaires complexes. La méthode MCKL fournit une approche classique pour ces modèles et le choix de la méthode d'analyse ne dépend plus nécessairement de la complexité du modèle.

[Traduit par la Rédaction]

Introduction

A major challenge of fitting fisheries models to time-series data has been to incorporate the combined effects of process and observation error in the framework of state-space models, which combine a stochastic model for population (state) dynamics with a stochastic model for observations or catches (Schnute 1994). The primary methods for fitting state-space models have been the Kalman Filter (KF) for classical analysis with linear models (Sullivan 1992; Pella 1993; Gudmundsson 1994), the "errors-in-variables" (EV) method, in which the joint likelihood of states and population dynamics parameters is maximized (Ludwig and Walters 1981; Ludwig et al. 1988; Richards et al. 1997), and the Bayesian approach, usually implemented by a Markov Chain Monte Carlo (MCMC) sample of the posterior distribution of states and parameters (McAllister et al. 1994; Meyer and Millar 1999; Schnute and Kronlund 2002).

From the point of view of asymptotic likelihood theory, which motivates both Bayesian and classical analysis with the assurance that more data provide better estimates on average, it is desirable to have methods that calculate the likelihood of population dynamics parameters by integrating over the state-space of population trajectories. The KF for classical analysis and MCMC posterior sampling for Bayesian analysis both accomplish this integration, but the KF is limited to linear models. Thus, a gap in available methodology has been the lack of methods to conduct classical analysis with nonlinear state-

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space models using likelihoods that integrate over the state-space (but see Kitagawa (1987) and de Valpine and Hastings (2002) for methods that do this for low-dimensional models). The EV method can use nonlinear models and has sometimes been treated as a classical maximum likelihood method, but it does not use an integrated likelihood and thus can have asymptotically biased parameter estimates and unknown confidence intervals (CIs).

In this paper, we evaluate and test the Monte Carlo Kernel Likelihood method (MCKL; de Valpine 2003, 2004) for classical likelihood analysis with nonlinear state-space models. MCKL estimates likelihoods with a weighted kernel density estimate of a Bayesian posterior sample, so it is as flexible as Bayesian MCMC methods and can be used as a complementary tool. Our purpose is to fill a gap in available methodology, not to weigh the merits of Bayesian and classical approaches for various applications. In many management contexts, Bayesian analysis may remain the method of choice, particularly as a way to provide state (stock) estimates that incorporate uncertainty in the population dynamics parameters (Punt and Hilborn 1997). MCKL can provide state distributions given particular population parameters (analogous to the KF; Schnute 1994), but the classical interpretation of parameters makes it harder to incorporate parameter uncertainty in as simple a way as the Bayesian approach, and state estimation is not a focus of this paper.

We motivate the need for the MCKL method by comparing integrated with nonintegrated likelihoods in a classical framework. The EV method maximizes the joint likelihood of population states and parameters without integration over the state-space. It has long been recognized that EV does not use classical likelihoods (e.g., Mendelssohn 1988; Schnute 1994; Millar and Meyer 2000) and can give biased estimates (Polacheck et al. 1993; Schnute and Richards 1995). However, EV has sometimes still been treated as if asymptotic likelihood theory applies, such as by estimating confidence intervals using the second derivatives of the joint likelihood to estimate a Fisher information matrix (Ludwig and Walters 1981; Richards et al. 1997; Otter Research 2000) and using information criteria for model selection. To compare EV with classical maximum likelihood in a simple case, we use a linear autoregressive model. Even for this model, EV gives asymptotically biased parameter estimates and inaccurate CIs. Although Bayesian methods are not a focus of this paper, our results comparing integrated with nonintegrated likelihoods also support Bayesian analysis, since a posterior MCMC sample effectively integrates over the state-space.

Our comparison of EV and likelihood approaches uses both theory and simulations. State-space (integrated) likelihood methods have generally been evaluated by applying them to one or a few real data sets (e.g., Freeman and Kirkwood 1994; Kimura et al. 1996; Millar and Meyer 2000). However, this approach has the shortcoming that for any single data set, it is impossible to know which method happens to provide a good inference. An alternative approach is to compare methods using many simulated time series. This reveals the distribution of estimation behavior for each method, which allows comparisons of bias, variance, and accuracy of CIs (or Bayesian confidence regions). This approach has been used by Ludwig et al. (1988), Ludwig and Walters (1989), and Polacheck et al. (1993) in comparison of EV and non-state-space methods and by de Valpine and Hastings (2002) for a state-space likelihood method. Here we use replicated simulations to compare state-space likelihood and EV methods for linear and nonlinear models, including a realistic example based on the cape hake (*Merluccius capensis* × *M. paradoxus*) data analyzed by Hilborn and Mangel (1997), although our simulations are limited by using only the single recorded catch data.

This paper is organized into four sections. First we introduce the state-space framework and the difference between classical (integrated) likelihoods and EV (nonintegrated) likelihoods. Then we present simulation and theoretical results for the linear, autoregressive model. Next we introduce the MCKL method, and finally we compare it with EV using the Namibian hake example. To allow comparison against a known benchmark, we first fit a Schaefer model to the hake data using MCKL, simulate many data sets using the estimated parameters and actual catch records, and then fit the simulated series with both MCKL and EV.

State-space framework

This introduction to the general state-space model framework draws on Kitagawa (1987), Harvey (1989), and Schnute (1994) and uses notation similar to that of Schnute (1994).

Denote the true state of the population at time t as a scalar or vector $\boldsymbol{X}_t$, which might be population size or a stage or age vector. Let $\boldsymbol{X}^t = (\boldsymbol{X}_1, ..., \boldsymbol{X}_t)$, and let T be the final observation time, so the entire series is $\boldsymbol{X}^T$. Let the stochastic model for the true population dynamics be

$$(1) \qquad \boldsymbol{X}_t = F(\boldsymbol{X}_{t-1}, v_{t-1})$$

where v_{t-1} is a random variable that provides the process error of the model. F might be a biomass dynamics model, a catch-at-age model, or some other fisheries model.

Denote the observation at time t as scalar or vector $\boldsymbol{Y}_t$, which might include total biomass, stage-structure or other information, and might aggregate or omit components of $\boldsymbol{X}_t$. Let $\boldsymbol{Y}^t = (\boldsymbol{Y}_1, ..., \boldsymbol{Y}_t)$. Denote the stochastic model for the observations as

$$(2) \qquad \boldsymbol{Y}_t = G(\boldsymbol{X}_t, \varepsilon_t)$$

where ε_t is a random variable that provides observation error. The model could also include control variables such as catch (Schnute 1994), but for simplicity we initially omit these from the notation.

The parameters of F and G will be denoted as a vector, $\boldsymbol{\Theta}$, which we want to estimate. In this paper we reserve the term parameter for quantities like fecundity, survival, growth rate, and the means and variances of the distributions of process and observation errors. For convenience, we do not use "parameter" to refer to unknown states (or process errors), as it is sometimes used in Bayesian analysis and EV analysis, so that we can discuss these two categories unambiguously.

Likelihoods vs. EV likelihoods

For a state-space model, the states and observations are jointly distributed random variables, and the likelihood is the marginal

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probability of the observations, integrated over all possible state trajectories. This is given by

$$(3)\quad L(\boldsymbol{\Theta}|\boldsymbol{Y}^T) = P(\boldsymbol{Y}^T|\boldsymbol{\Theta}) = \int P(\boldsymbol{Y}^T, \boldsymbol{X}^T|\boldsymbol{\Theta})\,\mathrm{d}\boldsymbol{X}^T = \int P(\boldsymbol{Y}^T|\boldsymbol{X}^T, \boldsymbol{\Theta})P(\boldsymbol{X}^T|\boldsymbol{\Theta})\,\mathrm{d}\boldsymbol{X}^T$$

where $P(\boldsymbol{Y}^T, \boldsymbol{X}^T|\boldsymbol{\Theta})$ is the probability density of states and observations given parameters, calculated using the models F and G. Equation 3 corresponds to the classical definition of likelihood to which the usual asymptotic (large-sample) theory applies. The high-dimensional integral over $\boldsymbol{X}^T$ cannot be solved analytically except in the simplest cases, such as by the KF if F and G are linear with additive Gaussian noises. Equation 3 here plays the role of eq. 2.9 of Schnute (1994). Classical likelihood analysis calls for maximizing $L(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$ over $\boldsymbol{\Theta}$ and estimating a confidence region, often approximated using asymptotic likelihood theory.

The EV likelihood handles the unknown states differently than the classical likelihood (eq. 3) in that it considers a single, specific trajectory of states. The EV likelihood is defined as the probability of a specific state trajectory, and the observations:

$$(4)\quad L^{\mathrm{EV}}(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T) = P(\boldsymbol{Y}^T, \boldsymbol{X}^T|\boldsymbol{\Theta}) = P(\boldsymbol{Y}^T|\boldsymbol{X}^T, \boldsymbol{\Theta})P(\boldsymbol{X}^T|\boldsymbol{\Theta})$$

Note that it equals the integrand of the likelihood (eq. 3) for the particular states $\boldsymbol{X}^T$. Equation 4 here plays the role of eq. 2.6 in Schnute (1994). EV model-fitting calls for maximizing $L^{\mathrm{EV}}(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T)$ over both $\boldsymbol{\Theta}$ and $\boldsymbol{X}^T$. It has become a standard part of EV analysis to assume a known constraint on the process and observation error variances, usually in the form of a known ratio of the two variances.

In Bayesian analysis for $\boldsymbol{\Theta}$, one can consider the posterior for states and parameters together

$$(5)\quad \pi(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T) = \frac{P(\boldsymbol{Y}^T|\boldsymbol{X}^T, \boldsymbol{\Theta})P(\boldsymbol{X}^T|\boldsymbol{\Theta})P(\boldsymbol{\Theta})}{P(\boldsymbol{Y}^T)}$$

as well as the posterior for parameters

$$(6)\quad \pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T) = \frac{P(\boldsymbol{Y}^T|\boldsymbol{\Theta})P(\boldsymbol{\Theta})}{P(\boldsymbol{Y}^T)}$$

which can be obtained by integrating (eq. 5) over the state dimensions:

$$(7)\quad \pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T) = \int \pi(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T)\,\mathrm{d}\boldsymbol{X}^T$$

In a Monte Carlo implementation, one obtains a sample of $(\boldsymbol{\Theta}, \boldsymbol{X}^T)$ points from $\pi(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T)$. Dropping the $\boldsymbol{X}^T$ dimensions represents a Monte Carlo approximation of the integral (eq. 7), so the remaining $\boldsymbol{\Theta}$ values are a sample from $\pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$.

The EV method has a complicated history of justification and application. EV originated as an improvement over simply ignoring either process or observation errors (Ludwig and Walters 1981; Collie and Sissenwine 1983; Ludwig et al. 1988). In that context, the requirement of assuming a known ratio of the two error variances (or some constraint on them) was better than just omitting one type of error. Ludwig and Walters (1981) referred to the approach simply as "maximum likelihood" and suggested that "conventional large-sample" results applied to it, including the use of a Fisher information matrix "describing the distribution of the maximum likelihood estimates." (They also proposed a Bayesian interpretation for EV and pointed out that the EV variance estimate is badly biased, which is an indication that conventional large-sample theory for classical likelihoods — including asymptotically unbiased estimates — does not apply.) In a major synthesis of the state-space framework, Schnute (1994) compared the EV and the KF approaches. He placed EV in a Bayesian framework as a highest-posterior-density estimate of (eq. 5), but he also raised serious cautions and pointed toward other Bayesian estimation possibilities (which have now become feasible with MCMC sampling; Schnute and Kronlund 2002).

While it was recognized that EV likelihoods are not classical likelihoods (e.g., Mendelssohn 1988; Schnute 1994; Millar and Meyer 2000), the practice of applying asymptotic likelihood theory to EV estimates became somewhat common. For example, Schnute and Richards (1995) used $-2 \log L^{\mathrm{EV}}(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T)$ as an "inference function", a quantity that corresponds to approximations from classical likelihood theory. Similarly, Richards et al. (1997) called the Hessian of the EV estimate for the entire state–parameter vector an "observed Fisher information matrix" and stated that "highest posterior density confidence regions correspond exactly to regions obtained by likelihood ratio tests." This practice is also reflected in the manual for AD Model Builder (Otter Research 2000), a software package widely used for fisheries analysis. The manual gives an example presenting the inverse Hessian as a "covariance matrix of model parameters", including states (Otter Research 2000, pp. 1–22). All of these papers and the AD Model Builder package have made valuable contributions to fisheries science, which we do not discuss here; our scope is limited to evaluating the application of likelihood theory to EV likelihoods.

Given the practice of applying classical likelihood theory to EV likelihoods, it is worth emphasizing that some of the most useful properties of classical and Bayesian analysis apply only to eqs. 3 and 6, respectively, not to eqs. 4 or 5. For example, in the limit as $T \to \infty$, $\pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$ is asymptotically Gaussian (with covariance that may be estimated using the Fisher information matrix from the Hessian matrix of second derivatives) and puts more and more weight on the true $\boldsymbol{\Theta}$, but the same does not hold for $\pi(\boldsymbol{\Theta}, \boldsymbol{X}^T|\boldsymbol{Y}^T)$ with respect to the true values of $\boldsymbol{\Theta}$ or $\boldsymbol{X}^T$. Our investigation of EV estimation represents a reevaluation of its justification starting from Schnute's (1994) cautionary note, but where he referred to "KF", we simply refer to "likelihood" methods to generalize the discussion to nonlinear and (or) non-Gaussian models.

Example 1: autoregressive (AR) model

How much do these theoretical issues matter in practice? In particular, how do EV estimates with likelihood-style approximate CIs compare to true maximum likelihood estimates and approximate CIs? We start with a first-order autoregressive

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process with additive Gaussian errors, which allows us to combine theoretical results and simulations. Although this model is simpler than realistic fisheries models, it allows us to test the theoretical comparison of EV and likelihood methods in the simplest case. Since likelihood theory addresses parameter estimation rather than state (or latent variable) estimation, we focus on parameter estimation. State estimates are often of great interest in fisheries analysis but are beyond the scope of this paper.

For the constraint on error variances that is required for the EV method, we use the correct value. This is highly unrealistic, but it emphasizes that even under the correct variance constraint, EV methods behave differently than true likelihood methods.

The states of the model, X_t, will have dynamics:

$$X_t = \mu + \rho(X_{t-1} - \mu) + v_{t-1} \quad (8)$$

Observations will be made from states with error:

$$Y_t = X_t + \varepsilon_t \quad (9)$$

Here μ is the mean population size, ρ is the correlation between X_t and X_{t-1}, and v_t and ε_t are independently (from each other and through time) and identically normally distributed, with means of 0 and variances of σ_v^2 and σ_ε^2, respectively. We denote parameters by $\boldsymbol{\Theta} = (\mu, \rho, \sigma_v^2, \sigma_\varepsilon^2)$, states by $\boldsymbol{X}^T = (X_1, ..., X_T)$, and observations by $\boldsymbol{Y}^T = (Y_1, ..., Y_T)$.

Fitting methods

Denote the EV likelihood as

$$L_\lambda^{\mathrm{EV}}(\boldsymbol{\Theta}_C, \boldsymbol{X}^T|\boldsymbol{Y}^T) = P_\lambda(\boldsymbol{Y}^T|\boldsymbol{X}^T, \boldsymbol{\Theta}_C)P_\lambda(\boldsymbol{X}^T|\boldsymbol{\Theta}_C) \quad (10)$$

where the parameters to be estimated are constrained to $\boldsymbol{\Theta}_C = (\mu, \rho, \sigma_\varepsilon^2)$, and the variance ratio

$$\lambda = \frac{\sigma_v^2}{\sigma_v^2 + \sigma_\varepsilon^2} \quad (11)$$

is assumed known. Note that $\boldsymbol{\Theta} = (\boldsymbol{\Theta}_C, \frac{\lambda}{1-\lambda}\sigma_\varepsilon^2)$. The assumption of known λ is awkward but has become a standard assumption for EV analysis.

For purposes of comparison to the EV method, we consider two versions of the classical likelihood. The first version assumes a known λ and will be called the constrained Kalman Filter (CKF). The second version assumes nothing about the error variances and is the KF. The CKF method shares the same parameter space as the EV method, so comparison of results from EV and CKF will reveal the difference between EV (non-integrated) likelihoods and true (integrated) likelihoods. Comparison between CKF and KF will reveal the difference between assuming λ known vs. unknown, respectively.

The CKF likelihood is given by

$$L_\lambda^{\mathrm{CKF}}(\boldsymbol{\Theta}_C|\boldsymbol{Y}^T) = \int P_\lambda(\boldsymbol{Y}^T|\boldsymbol{X}^T, \boldsymbol{\Theta}_C)P_\lambda(\boldsymbol{X}^T|\boldsymbol{\Theta}_C)\mathrm{d}\boldsymbol{X}^T \quad (12)$$

and the KF likelihood is given by

$$L^{\mathrm{KF}}(\boldsymbol{\Theta}|\boldsymbol{Y}^T) = \int P(\boldsymbol{Y}^T|\boldsymbol{X}^T, \boldsymbol{\Theta})P(\boldsymbol{X}^T|\boldsymbol{\Theta})\,\mathrm{d}\boldsymbol{X}^T \quad (13)$$

The steps for KF calculations are straightforward and are given in many sources, including Harvey (1989, 1993) and Schnute (1994). For the simple example here, equivalent calculations are even easier using the full multivariate normal distribution of $\boldsymbol{Y}^T$ (Appendix A).

Theoretical predictions for the AR model

Several analytical results on L^{EV} for the AR model are presented in Appendix A, with log likelihoods denoted $l = \log(L)$, and can be summarized by the following: (*i*) As the amount of data increases, l^{EV} parameter estimates do not converge to the true parameters (i.e., they are asymptotically biased), even if λ is correct. l^{EV} differs from l^{KF} and l^{CKF}, which do converge to the true parameters (if λ is correct for l^{CKF}). (*ii*) l^{KF} and l^{EV} converge as observation error variance becomes small, so the asymptotic bias of l^{EV} goes to zero as observation error variance goes to zero. (*iii*) EV systematically underestimates the error variances. (*iv*) For EV, approximate CIs based on likelihood-type approximations are not accurate and will become less accurate as the amount of data increases, even if λ is correct. As the amount of data increases, the Hessian will be more sharply peaked around asymptotically incorrect parameters, so EV will offer increasingly specious certainty about parameters increasingly certain to be incorrect.

Simulations for the AR model

We simulated 200 data sets under every combination of $\rho = (-0.25, 0.25, 0.75)$, $\lambda = (0.25, 0.5, 0.75)$, and $n = (40, 200)$. In every case, the variances were chosen to keep constant the stationary variance in observations:

$$\sigma_y^2 = \frac{\sigma_v^2}{1-\rho^2} + \sigma_\varepsilon^2 \quad (14)$$

We used $\sigma_y^2 = 20$ for all simulations. The shorter series lengths ($n = 40$) are optimistic for well-studied fisheries, while the longer lengths allow comparison with asymptotic results. We used $\mu = 100$ for all simulations.

For every simulated data set, we estimated parameters using EV, CKF, and KF. For EV and CKF, we used the correct value of λ. We also calculated the likelihood-based, approximate p value of the true parameters for each method and reported the percentage of rejections of the true parameters compared with the expected number of rejections under asymptotic likelihood theory for Type I error rates of $\alpha = 0.05, 0.10, 0.25$, and 0.50.

Under asymptotic likelihood theory, –2 log ratio of the true parameter likelihood to the estimated parameter likelihood is distributed as χ_d^2, where d is the number of estimated parameters. For KF, we used this result to estimate p values for the true parameters. For EV and CKF, we used the related result that $(\boldsymbol{\Theta} - \hat{\boldsymbol{\Theta}})'\hat{\mathbf{I}}(\hat{\boldsymbol{\Theta}})(\boldsymbol{\Theta} - \hat{\boldsymbol{\Theta}}) \sim \chi_d^2$, where $\hat{\mathbf{I}}(\hat{\boldsymbol{\Theta}})$ is the estimated inverse covariance matrix of $\hat{\boldsymbol{\Theta}}$, calculated as the Hessian of the log-likelihood surface (i.e., the matrix of second derivatives) (e.g., Richards et al. 1997; Severini 2000). For EV we used the estimated inverse covariance matrix of only the parameter dimensions (i.e., we inverted the state–parameter Hessian to obtain the estimated state–parameter covariance matrix, extracted the estimated covariance of the parameter dimensions, and inverted that covariance matrix to estimate p values for the true parameters). For EV in a linear regression problem,

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Table 1. Mean, standard deviation (Std. Dev.), and root mean squared error (RMSE) of $\hat{\rho}$ in the autoregressive (AR) linear model example.

	$\rho = -0.25$			$\rho = 0.25$			$\rho = 0.75$		
	$\lambda = 0.25$	$\lambda = 0.50$	$\lambda = 0.75$	$\lambda = 0.25$	$\lambda = 0.50$	$\lambda = 0.75$	$\lambda = 0.25$	$\lambda = 0.50$	$\lambda = 0.75$
EV, $n = 40$									
Mean	−0.577	−0.423	−0.309	0.475	0.303	0.239	0.865	0.779	0.734
Std. Dev.	0.548	0.466	0.234	0.623	0.477	0.245	0.136	0.189	0.140
RMSE	0.638	0.497	0.241	0.663	0.480	0.245	0.179	0.192	0.141
CKF, $n = 40$									
Mean	−0.202	−0.243	−0.234	0.0527	0.158	0.176	0.536	0.589	0.641
Std. Dev.	0.363	0.275	0.186	0.401	0.262	0.183	0.332	0.203	0.155
RMSE	0.367	0.275	0.187	0.447	0.277	0.197	0.395	0.259	0.189
KF, $n = 40$									
Mean	−0.323	−0.358	−0.307	0.244	0.289	0.279	0.528	0.600	0.637
Std. Dev.	0.261	0.286	0.257	0.319	0.283	0.262	0.344	0.199	0.177
RMSE	0.271	0.306	0.263	0.319	0.286	0.263	0.409	0.249	0.210
EV, $n = 200$									
$\lim_{n\to\infty} \hat{\rho}$	−0.830	−0.595	−0.352	0.830	0.595	0.352	0.924	0.882	0.828
Mean	−0.820	−0.548	−0.345	0.815	0.553	0.320	0.921	0.865	0.808
Std. Dev.	0.122	0.201	0.103	0.124	0.157	0.116	0.0189	0.0329	0.0383
RMSE	0.583	0.359	0.141	0.579	0.341	0.135	0.172	0.120	0.0692
CKF, $n = 200$									
Mean	−0.238	−0.245	−0.251	0.201	0.233	0.231	0.720	0.719	0.728
Std. Dev.	0.221	0.133	0.0836	0.216	0.116	0.0908	0.0764	0.0635	0.0495
RMSE	0.222	0.133	0.0836	0.221	0.117	0.0928	0.0821	0.0705	0.0541
KF, $n = 200$									
Mean	−0.305	−0.362	−0.328	0.319	0.359	0.302	0.698	0.712	0.724
Std. Dev.	0.266	0.260	0.190	0.275	0.250	0.185	0.137	0.101	0.0657
RMSE	0.271	0.283	0.206	0.283	0.273	0.192	0.146	0.108	0.0705

Note: Column headers indicate parameters used for simulation. Row sections give results for different fitting methods (EV, errors-in-variables; KF, Kalman Filter; CKF, constrained Kalman Filter) and different series lengths. Calculated asymptotic value is shown as $\lim_{n\to\infty} \hat{\rho}$.

Schnute et al. (1990) showed that one-half the usual likelihood ratio calculation (or related Hessian calculation) gave the usual χ^2_d result, and Schnute (1994) raised the question of whether a similar result would apply to EV for time series, so we also calculated p values based on this quantity ("EV adjusted").

Results for the AR simulations

Simulations confirm the theoretical predictions about EV, CKF, and KF (Tables 1, 2; Figs. 1, 2). In nearly every case, CKF has lower root mean squared error ($\text{RMSE} = \sqrt{\text{bias}^2 + \text{variance}}$) than EV. For EV, more data often lead to increased bias, as the estimates converge to an asymptotically biased estimate, while for CKF more data lead to decreased bias. The arrows in Figs. 1 and 2 and the line "$\lim_{n\to\infty}(\cdot)$" in Tables 1 and 2 give the theoretical asymptotic parameter estimates for EV, obtained computationally as described in Appendix A. The means of the $n = 200$ results are close to these values. The only cases where EV has smaller RMSE than CKF are the ρ estimates with $n = 40$ and $\lambda = 0.75$, but even in these cases, EV estimates converge away from the true values as n increases, while CKF estimates converge toward the true values. For both $n = 40$ and $n = 200$, EV systematically underestimates the error variances, consistent with Ludwig and Walters (1981), Schnute and Kronlund (2002), and results therein.

Likelihood-based p values for EV are not even approximately accurate (Table 3). For example, using the χ^2_d cutoff that asymptotically rejects the true parameters with frequency 5%, EV rejected the true parameters in 99% of simulations, while CKF rejected them in 11% of simulations. The EV-adjusted results calculated from twice the usual test statistic are also very inaccurate. For CKF, p value percentiles differ from the asymptotic expectation but may be considered to be a useful approximation.

In terms of both parameter estimates and p values, KF performs substantially worse than CKF owing to its difficulty estimating error variances. Nevertheless, its confidence regions (as reflected by p value results) offer a useful approximation of uncertainty. For example, although KF does not estimate error variances well, it does include the true parameters in a putative 95% confidence region roughly 99% of the time (across the range of simulation parameters). In summary, classical likelihoods outperform EV likelihoods (CKF vs. EV), while it remains true that separating process and observation error is an inherently difficult problem (KF vs. CKF).

Having compared classical and EV likelihoods with theory and simulations, we reevaluate Schnute's (1994) proposed justification of the EV method from a Bayesian point of view, which may be summarized as follows: (*i*) Bayesian analysis treats both states and parameters as random variables. (In a fre-

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Table 2. Mean, standard deviation (Std. Dev.), and root mean squared error (RMSE) for $\hat{\alpha}_1$ in the autoregressive (AR) linear model example.

	$\rho = -0.25$			$\rho = 0.25$			$\rho = 0.75$		
	$\lambda = 0.25$ $\sigma_\varepsilon = 3.84$	$\lambda = 0.50$ $\sigma_\varepsilon = 3.11$	$\lambda = 0.75$ $\sigma_\varepsilon = 2.18$	$\lambda = 0.25$ $\sigma_\varepsilon = 3.84$	$\lambda = 0.50$ $\sigma_\varepsilon = 3.11$	$\lambda = 0.75$ $\sigma_\varepsilon = 2.18$	$\lambda = 0.25$ $\sigma_\varepsilon = 3.37$	$\lambda = 0.50$ $\sigma_\varepsilon = 2.47$	$\lambda = 0.75$ $\sigma_\varepsilon = 1.60$
EV, $n = 40$									
Mean	2.54	2.08	1.48	2.56	2.08	1.49	2.28	1.68	1.08
Std. Dev.	0.303	0.262	0.163	0.301	0.235	0.165	0.266	0.217	0.129
RMSE	1.33	1.07	0.723	1.32	1.05	0.715	1.12	0.819	0.534
CKF, $n = 40$									
Mean	3.69	2.98	2.10	3.70	2.98	2.11	3.32	2.42	1.53
Std. Dev.	0.429	0.372	0.230	0.429	0.333	0.234	0.390	0.314	0.184
RMSE	0.454	0.395	0.245	0.453	0.356	0.245	0.392	0.318	0.194
KF, $n = 40$									
Mean	2.48	2.82	3.41	2.17	2.64	3.12	2.39	2.59	2.75
Std. Dev.	1.89	1.78	1.38	1.95	1.83	1.54	1.21	0.987	0.624
RMSE	2.33	1.80	1.84	2.57	1.89	1.80	1.56	0.994	1.31
EV, $n = 200$ + $\lim_{n\to\infty} \hat{\alpha}_1$	2.62	2.17	1.54	2.62	2.17	1.54	2.32	1.72	1.12
Mean	2.62	2.15	1.53	2.62	2.16	1.53	2.31	1.71	1.12
Std. Dev.	0.142	0.116	0.0816	0.126	0.113	0.0744	0.112	0.0875	0.0555
RMSE	1.23	0.963	0.660	1.23	0.958	0.654	1.07	0.761	0.481
CKF, $n = 200$									
Mean	3.82	3.09	2.16	3.81	3.09	2.17	3.34	2.46	1.59
Std. Dev.	0.201	0.164	0.115	0.182	0.160	0.105	0.165	0.127	0.0791
RMSE	0.202	0.165	0.116	0.184	0.161	0.106	0.166	0.127	0.0793
KF, $n = 200$									
Mean	2.65	2.89	3.51	2.40	2.88	3.55	2.09	2.50	2.79
Std. Dev.	1.79	1.48	1.09	1.80	1.45	1.06	0.664	0.464	0.351
RMSE	2.14	1.50	1.72	2.30	1.47	1.73	1.44	0.465	1.24

Note: Column headers indicate parameters used for simulation. Row sections give results for different fitting methods (EV, errors-in-variables; KF, Kalman Filter; CKF, constrained Kalman Filter) and different series lengths. Calculated asymptotic value is shown as $\lim_{n\to\infty} \hat{\rho}$.

quentist view, parameters are not random variables, but states are.) (*ii*) Bayesian analysis sometimes estimates parameters by finding the maximum posterior probability. (*iii*) If states and parameters are both random variables, they can be estimated the same way (i.e., by maximizing their joint posterior). For an uninformative prior, this is the same as maximizing the EV likelihood, which is the EV method. The reasoning is summarized by Schnute's (1994, p. 1680) statement that "the Bayes framework easily allows the random states $\mathbf{X}_t$ to be considered parameters, so that the posterior (2.11) [here eq. 5] is meaningful without integration."

As shown for even a simple linear model, a weakness in this reasoning is that likelihood theory does not apply to joint estimates of states and parameters. The breakdown in the proposed justification is that the usual likelihood properties of Bayesian and classical inference apply only to the parameters, not the states, so that using the joint state–parameter Bayesian posterior as a justification for the EV likelihood subtly drops the applicability of standard likelihood theory.

Monte Carlo state-space likelihoods

Next we present a method for classical maximum likelihood estimation with nonlinear state-space models, based on Monte Carlo approximations of the likelihood integral (de Valpine 2003, 2004). Several algorithms have been developed for this problem in non-fisheries contexts, including basic Monte Carlo integration (Durbin and Koopman 1997, 2000), Monte Carlo expectation maximization (Wei and Tanner 1990; Chan and Ledolter 1995; Booth and Hobert 1999), maximization based on local Monte Carlo likelihood ratio estimation (Geyer and Thompson 1992; McCulloch 1997), and "particle filter" methods (Gordon et al. 1993; Pitt and Shephard 1999; Hürzeler and Kunsch 2001). For the case of stage-structured population models, de Valpine (2004) found that a new method (MCKL) was more efficient than these others. In addition, MCKL has the advantage that it estimates classical likelihoods from a Monte Carlo Bayesian posterior, so that the same computational work can be used for both Bayesian and classical analyses. We present the basic rationale behind the MCKL method; theoretical convergence and accuracy are considered by de Valpine (2004).

Consider the general problem of estimating the likelihood, $L(\boldsymbol{\Theta}) \equiv P(\mathbf{Y}^T|\boldsymbol{\Theta})$, from a Monte Carlo sample of the posterior $\pi(\boldsymbol{\Theta}|\mathbf{Y}^T)$, denoted by $[\boldsymbol{\Theta}^{(j)}]_{j=1}^m$ for a sample with m points. The difficulty is that $\pi(\boldsymbol{\Theta}|\mathbf{Y}^T) \propto L(\boldsymbol{\Theta})P(\boldsymbol{\Theta})$, where $P(\boldsymbol{\Theta})$ is the prior, so we need to divide by $P(\boldsymbol{\Theta})$ after Monte Carlo sampling. (We cannot assume that $P(\boldsymbol{\Theta})$ can be chosen as flat everywhere because then $\pi(\boldsymbol{\Theta}|\mathbf{Y}^T)$ may not have finite integral, leading MCMC algorithms to fail.)

To solve this problem, MCKL uses two tools of Monte Carlo

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Fig. 1. Estimates of ρ for the autoregressive (AR) model. Box plots of 200 point estimates of ρ from simulated time series of length 40 for each of nine simulation parameter values ($3\lambda \times 3\rho$ values: (*a*) $\rho = -0.25$, (*b*) $\rho = 0.25$, (*c*) $\rho = 0.75$). EV, errors-in-variables; CKF, constrained Kalman Filter; KF, Kalman Filter. Boxes show 25th, 50th, and 75th percentiles; dashed whiskers extend to the farthest value within 1.5 times the interquartile range; dots show all remaining values; °, mean; dotted horizontal line, true value. Arrows for EV shows asymptotic value (i.e., the bias given infinite data). (Asymptotic value for CKF and KF is the true value.)

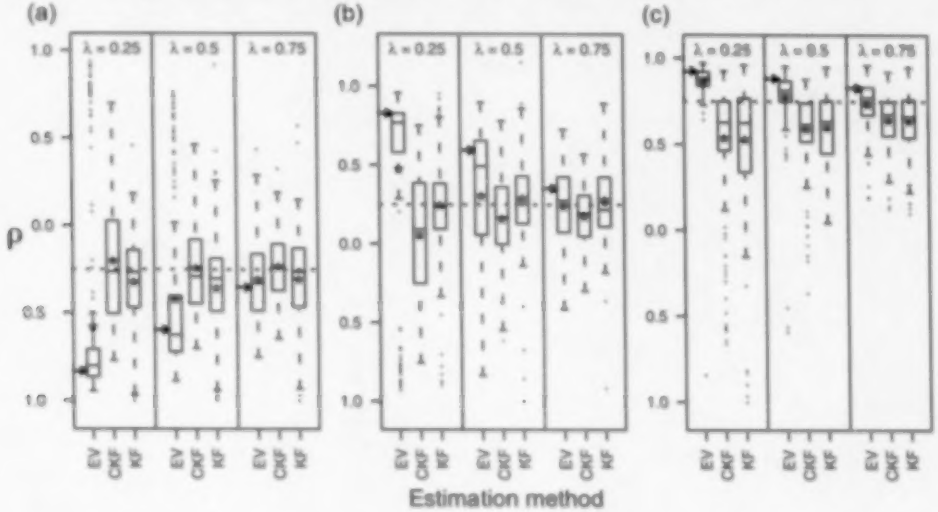
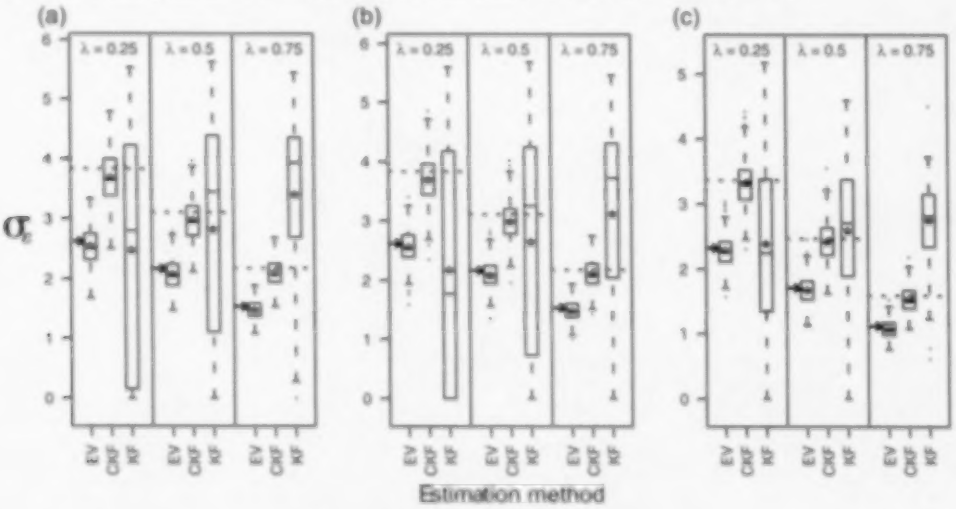


Fig. 2. Estimates of σ_ε for the autoregressive (AR) model. Box plots of 200 point estimates of ρ from simulated time series of length 40 for each of nine simulation parameter values ($3\lambda \times 3\rho$ values: (*a*) $\rho = -0.25$, (*b*) $\rho = 0.25$, (*c*) $\rho = 0.75$). EV, errors-in-variables; CKF, constrained Kalman Filter; KF, Kalman Filter. Boxes show 25th, 50th, and 75th percentiles; dashed whiskers extend to the farthest value within 1.5 times the interquartile range; dots show all remaining values; °, mean; dotted horizontal line, true value. Arrows for EV shows asymptotic value (i.e., the bias given infinite data). (Asymptotic value for CKF and KF is the true value.)



methodology: kernel density estimation and importance sampling. If the goal was to estimate values of $\pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$, this would be a standard problem of density estimation from a sample, and we could use kernel density estimation alone. We define $K_{\mathbf{h}}(\boldsymbol{\Theta})$ to be a multivariate normal density function (the kernel) with mean zero and a diagonal covariance matrix with values $(h_1^2, h_2^2, \ldots, h_d^2)$. (The choice of diagonal covariance matrix is for simplicity of notation only.) A kernel density estimate of $\pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$ is

$$\hat{\pi}(\boldsymbol{\Theta}|\boldsymbol{Y}^T) \equiv \frac{1}{m}\sum_{j=1}^{m} K_{\mathbf{h}}[\boldsymbol{\Theta} - \boldsymbol{\Theta}^{(j)}] \quad (15)$$

Intuitively, $\hat{\pi}(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$ is a weighted average of the number of points in the Monte Carlo sample that are near $\boldsymbol{\Theta}$, with weights determined by the kernel. Choice of $\mathbf{h}$ is determined by a balance

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Table 3. Confidence interval (CI) performance of fitting methods for the autoregressive (AR) linear model example.

	$\rho = -0.25$			$\rho = 0.25$			$\rho = 0.75$		
	$\lambda = 0.25$	$\lambda = 0.50$	$\lambda = 0.75$	$\lambda = 0.25$	$\lambda = 0.50$	$\lambda = 0.75$	$\lambda = 0.25$	$\lambda = 0.50$	$\lambda = 0.75$
Expected percentile	$\sigma_\varepsilon = 3.84$	$\sigma_\varepsilon = 3.11$	$\sigma_\varepsilon = 2.18$	$\sigma_\varepsilon = 3.84$	$\sigma_\varepsilon = 3.11$	$\sigma_\varepsilon = 2.18$	$\sigma_\varepsilon = 3.37$	$\sigma_\varepsilon = 2.47$	$\sigma_\varepsilon = 1.60$
EV, $n = 40$									
0.05	0.990	0.970	0.985	0.990	0.980	0.975	0.955	0.950	0.975
0.10	0.990	0.975	0.995	0.995	0.985	0.985	0.975	0.960	0.985
0.25	0.995	0.980	0.995	0.995	0.995	0.995	0.985	0.975	0.985
0.50	1	1	1	0.995	0.995	1	0.990	0.990	0.990
EV adjusted, $n = 40$									
0.05	0.950	0.920	0.885	0.940	0.915	0.835	0.840	0.825	0.820
0.10	0.975	0.930	0.920	0.970	0.955	0.885	0.890	0.865	0.880
0.25	0.990	0.970	0.955	0.990	0.975	0.955	0.930	0.940	0.970
0.50	0.990	0.975	0.995	0.995	0.985	0.990	0.985	0.970	0.985
CKF, $n = 40$									
0.05	0.110	0.090	0.065	0.135	0.090	0.055	0.100	0.115	0.145
0.10	0.150	0.135	0.105	0.185	0.125	0.100	0.150	0.165	0.205
0.25	0.270	0.245	0.195	0.280	0.225	0.170	0.235	0.280	0.340
0.50	0.410	0.455	0.360	0.460	0.425	0.370	0.395	0.480	0.500
KF, $n = 40$									
0.05	0.015	0.015	0.005	0.025	0.035	0.010	0.020	0.035	0.025
0.10	0.045	0.040	0.015	0.025	0.050	0.020	0.045	0.070	0.055
0.25	0.095	0.120	0.105	0.075	0.110	0.070	0.140	0.175	0.165
0.50	0.220	0.305	0.260	0.205	0.245	0.220	0.340	0.415	0.430
EV, $n = 200$									
0.05	1	1	1	1	1	1	1	1	1
0.10	1	1	1	1	1	1	1	1	1
0.25	1	1	1	1	1	1	1	1	1
0.50	1	1	1	1	1	1	1	1	1
EV adjusted, $n = 200$									
0.05	1	1	1	1	1	1	1	1	1
0.10	1	1	1	1	1	1	1	1	1
0.25	1	1	1	1	1	1	1	1	1
0.50	1	1	1	1	1	1	1	1	1
CKF, $n = 200$									
0.05	0.060	0.045	0.050	0.065	0.015	0.035	0.015	0.045	0.015
0.10	0.110	0.085	0.070	0.075	0.035	0.050	0.035	0.120	0.075
0.25	0.255	0.205	0.165	0.175	0.135	0.125	0.120	0.185	0.190
0.50	0.410	0.395	0.355	0.355	0.320	0.330	0.320	0.355	0.365
KF, $n = 200$									
0.05	0.010	0.025	0.025	0.005	0.020	0.015	0.005	0.030	0.015
0.10	0.040	0.075	0.040	0.015	0.030	0.035	0.035	0.060	0.050
0.25	0.105	0.155	0.125	0.085	0.070	0.090	0.105	0.205	0.125
0.50	0.285	0.355	0.270	0.235	0.265	0.265	0.315	0.380	0.380

Note: Column headers indicate parameters used for simulation. Row sections give results for different fitting methods (EV, errors-in-variables; KF, Kalman Filter; CKF, constrained Kalman Filter) and different series lengths. Rows are labeled by the p value cutoff that was used (0.05, 0.10, 0.25, 0.50). Table values give percent of simulations in which the true parameters were rejected using the given cutoff. For example, EV rejected the true parameters over 95% of the time when a nominal rejection percentage of only 5% was chosen.

between bias and variance. Small $\mathbf{h}$ gives low bias but high variance, and vice-versa.

A more general view of eq. 15 is that it is a Monte Carlo approximation to the convolution (or smoothing) integral of $K_{\mathbf{h}}$ and π, which is defined by

$$\hat{\pi}(\boldsymbol{\Theta}|\boldsymbol{Y}^T) = \int K_{\mathbf{h}}(\boldsymbol{\Theta} - \boldsymbol{\phi})\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)\,d\boldsymbol{\phi} \quad (16)$$

This integral can be interpreted as the average of $K_{\mathbf{h}}(\boldsymbol{\Theta} - \boldsymbol{\phi})$ over the distribution $\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)$. A natural way to estimate an average over a distribution is to use a sample of points from the distribution and calculate the average directly, which explains why eq. 15 is an estimate of eq. 16.

The next step is to adjust the kernel density estimate to recover $L(\boldsymbol{\Theta})$ instead of $\pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$ (i.e., to divide out the prior after the Monte Carlo sample has been obtained). There must still be some smoothing, since we are estimating a density from a Monte Carlo sample, so a goal is to estimate the convolution

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Table 4. Mean, standard deviation (Std. Dev.), and root mean squared error (RMSE) of $\log(\tilde{r})$ for the hake (*Merluccius capensis* × *M. paradoxus*) example using errors-in-variables (EV; λ known) and Monte Carlo Kernel Likelihood (MCKL; λ known vs. unknown).

	Parameter used for simulation		
	$\Theta_{.01}$[a]	$\Theta_{.05}$[b]	$\Theta_{.10}$[c]
EV (λ known)			
Mean	−1.78	−1.51	−1.41
Std. Dev.	0.723	0.612	0.336
RMSE	0.918	0.726	0.525
MCKL (λ known)			
Mean	−1.17	−1.12	−1.12
Std. Dev.	0.523	0.461	0.302
RMSE	0.524	0.461	0.320
MCKL (λ unknown)			
Mean	−1.25	−1.18	−1.13
Std. Dev.	0.583	0.490	0.331
RMSE	0.584	0.495	0.352

[a]$\log(r) = -1.21$.
[b]$\log(r) = -1.18$.
[c]$\log(r) = -1.01$.

Table 5. Mean, standard deviation (Std. Dev.), and root mean squared error (RMSE) for $\log(\tilde{q})$ for the hake (*Merluccius capensis* × *M. paradoxus*) example using errors-in-variables (EV; λ known) and Monte Carlo Kernel Likelihood (MCKL; λ known vs. unknown).

	Parameter used for simulation		
	$\Theta_{.01}$[a]	$\Theta_{.05}$[b]	$\Theta_{.10}$[c]
EV (λ known)			
Mean	−7.49	−7.24	−7.22
Std. Dev.	0.379	0.555	0.399
RMSE	0.601	0.844	0.684
MCKL (λ known)			
Mean	−8.07	−7.98	−7.91
Std. Dev.	0.351	0.346	0.260
RMSE	0.367	0.361	0.291
MCKL (λ unknown)			
Mean	−8.04	−7.98	−7.90
Std. Dev.	0.359	0.361	0.291
RMSE	0.367	0.377	0.316

[a]$\log(q) = -7.96$.
[b]$\log(q) = -7.87$.
[c]$\log(q) = -7.78$.

of the likelihood (rather than the posterior), defined by

$$\tilde{L}(\boldsymbol{\Theta}) \equiv \int K_{\mathbf{h}}(\boldsymbol{\Theta} - \boldsymbol{\phi}) L(\boldsymbol{\phi})\, \mathrm{d}\boldsymbol{\phi} \tag{17}$$

This is equivalent to

$$\tilde{L}(\boldsymbol{\Theta}) = \int K_{\mathbf{h}}(\boldsymbol{\Theta} - \boldsymbol{\phi}) \frac{L(\boldsymbol{\phi})}{\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)} \pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)\, \mathrm{d}\boldsymbol{\phi} \tag{18}$$

which is the average of $K_{\mathbf{h}}(\boldsymbol{\Theta} - \boldsymbol{\phi}) \frac{L(\boldsymbol{\phi})}{\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)}$ over the distribution $\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)$. Substituting $\frac{L(\boldsymbol{\phi})}{\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)} = \frac{P(\boldsymbol{Y}^T)}{P(\boldsymbol{\phi})}$, the natural Monte Carlo estimate of eq. 18 is

$$\hat{L}(\boldsymbol{\Theta}) \equiv \frac{P(\boldsymbol{Y}^T)}{m} \sum_{j=1}^{m} \frac{K_{\mathbf{h}}[\boldsymbol{\Theta} - \boldsymbol{\Theta}^{(j)}]}{P[\boldsymbol{\Theta}^{(j)}]} \tag{19}$$

The step of multiplying and dividing by $\pi(\boldsymbol{\phi}|\boldsymbol{Y}^T)$ in eq. 18, so that each sample point $\boldsymbol{\Theta}^{(j)} \sim \pi(\boldsymbol{\Theta}|\boldsymbol{Y}^T)$ is weighted by $1/P[\boldsymbol{\Theta}^{(j)}]$ in eq. 19, is known as importance sampling. Finally, the unknown constant $P(\boldsymbol{Y}^T)$ can be ignored for maximization of eq. 19 over $\boldsymbol{\Theta}$, giving the MCKL estimate:

$$\hat{L}_{\mathrm{U}}(\boldsymbol{\Theta}) \equiv \frac{1}{m} \sum_{j=1}^{m} \frac{K_{\mathbf{h}}[\boldsymbol{\Theta} - \boldsymbol{\Theta}^{(j)}]}{P[\boldsymbol{\Theta}^{(j)}]} \tag{20}$$

where subscript U indicates unnormalized. In summary, eq. 20 is a weighted, importance-sampled, kernel density estimate of the likelihood up to an unknown constant, where the Bayesian posterior serves as the importance sampling density. For some analyses (such as model selection using information criteria), a normalized likelihood is necessary. $P(\boldsymbol{Y}^T)$ may be estimated by methods such as importance sampling or particle filtering that would be too slow for maximization but are feasible for a single calculation, such as at the maximum of $\hat{L}_{\mathrm{U}}(\boldsymbol{\Theta})$.

Since eq. 20 is an approximation of a convolved likelihood, it raises several issues of convergence, accuracy, and practical implementation. In terms of theoretical convergence, standard results for kernel density estimation give that $\lim_{m\to\infty} \hat{L}_{\mathrm{U}}(\boldsymbol{\Theta}) = \tilde{L}(\boldsymbol{\Theta})/P(\boldsymbol{Y}^T)$, and $\lim_{\mathbf{h}\to 0} \tilde{L}(\boldsymbol{\Theta}) = L(\boldsymbol{\Theta})$ (Silverman 1986; Scott 1992). For these results to hold here, the following conditions are required (de Valpine 2004). We define $\psi_j(\boldsymbol{\Theta}) \equiv K_{\mathbf{h}}[\boldsymbol{\Theta} - \boldsymbol{\Theta}^{(j)}]/P[\boldsymbol{\Theta}^{(j)}]$, and we define $\nabla\psi_j(\boldsymbol{\Theta})$ and $\nabla^2\psi_j(\boldsymbol{\Theta})$ to be the vector of first derivatives and matrix of second derivatives with respect to $\boldsymbol{\Theta}$, respectively. Then it is required that $E[\nabla^2\psi_j(\boldsymbol{\Theta}_{\mathbf{h}})]$ be invertible and $E\{[\nabla\psi_j(\boldsymbol{\Theta}_{\mathbf{h}})][\nabla\psi_j(\boldsymbol{\Theta}_{\mathbf{h}})]^T\}$ exist, where $\boldsymbol{\Theta}_{\mathbf{h}}$ is the maximum of $\tilde{L}$, and the expectations are with respect to the sampling distribution $\pi[\boldsymbol{\Theta}^{(j)}|\boldsymbol{Y}^T]$.

Implementation of MCKL requires two steps: (*i*) obtain an MCMC sample of the Bayesian posterior, and (*ii*) maximize eq. 20. MCMC sampling methodology is advancing quickly. Equation 20 is straightforward to program, but one must choose sample size (m) and multivariate smoothing bandwidth ($\mathbf{h}$). Optimal choice of bandwidth in standard kernel density estimation is a highly developed topic, but the current problem is different because of the inverse-prior weighting and the possibility of simulating additional points for the smoothing sample. de Valpine (2004) gives an approach to choosing $\mathbf{h}$ based on the approximately Gaussian shape of the log likelihood surface near its maximum. One can even choose $\mathbf{h}$ manually, based on inspection of estimated likelihood surfaces, until better-developed, automated approaches become available. In addition, it is possible to improve accuracy after obtaining an initial maximum likelihood estimate by using a new prior, with heavier weight near the maximum, and obtaining a second MCMC sample that

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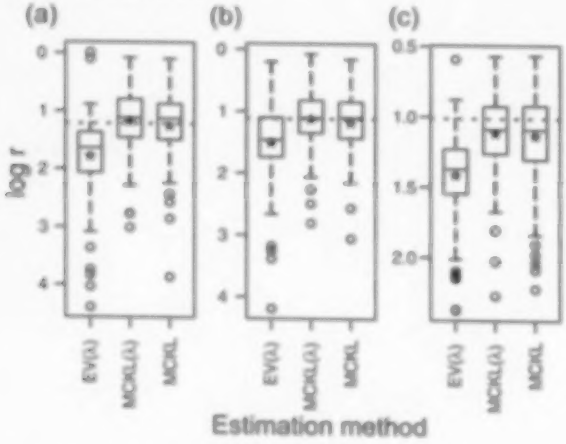
Fig. 3. Estimates of log(r) for the hake (*Merluccius capensis* × *M. paradoxus*) model. Box plots of 100 point estimates of log(r) for each of three simulation parameters: (*a*) $\sigma_\varepsilon = 0.01$, (*b*) $\sigma_\varepsilon = 0.05$, (*c*) $\sigma_\varepsilon = 0.10$ (see text). EV, errors-in-variables; MCKL, Monte Carlo Kernel Likelihood. (λ) indicates that λ is assumed correctly known.



gives greater MCKL accuracy near the maximum ("zooming in"; de Valpine 2004).

Note that the density estimation must be conducted in the same coordinates as the sampling; transformation to other coordinates before density estimation would introduce a correction based on the determinant of the Jacobian of the transformation (as in standard probability theory), which we do not give here. It is safe, however, to use a linear transformation, such as principle components, since the Jacobian is simply a constant.

In fisheries applications, the distribution of states, particularly of the last state, given parameters may be of great interest. This distribution may be obtained in one of two ways. First, one may include the final state dimension in the kernel-smoothing integral after estimating the maximum likelihood parameters $\hat{\boldsymbol{\Theta}}$. For example, one would first estimate $\hat{\boldsymbol{\Theta}}$ and then use eq. 20 with the X_T dimension included to estimate $P(X_T|\hat{\boldsymbol{\Theta}})$. Second, one could run the MCMC algorithm for states only with parameters fixed at $\hat{\boldsymbol{\Theta}}$ to obtain a sample from $P(X_T|\hat{\boldsymbol{\Theta}})$ (obtaining this sample will be much faster than obtaining the joint state–parameter sample). As noted in the Introduction, management-oriented incorporation of parameter uncertainty into estimates of the state distribution still provides strong motivation for a Bayesian approach. This paper fills in the gap of classical likelihood methods for state-space models but does not conflict with Bayesian approaches. Having indicated how one would estimate state distributions using MCKL, we defer further comparison of state estimates to further research and focus here on comparison of parameter estimates using different methods.

Example 2: hake

To illustrate MCKL and compare it with EV in a real but relatively simple fisheries problem, we use the hake data (annual catch and abundance index from 1965 to 1987) and Schaefer

Fig. 4. Estimates of log(q) for the hake (*Merluccius capensis* × *M. paradoxus*) model. Box plots of 100 point estimates of log(q) for each of three simulation parameters: (*a*) $\sigma_\varepsilon = 0.01$, (*b*) $\sigma_\varepsilon = 0.05$, (*c*) $\sigma_\varepsilon = 0.10$ (see text). EV, errors-in-variables; MCKL, Monte Carlo Kernel Likelihood. (λ) indicates that λ is assumed correctly known.

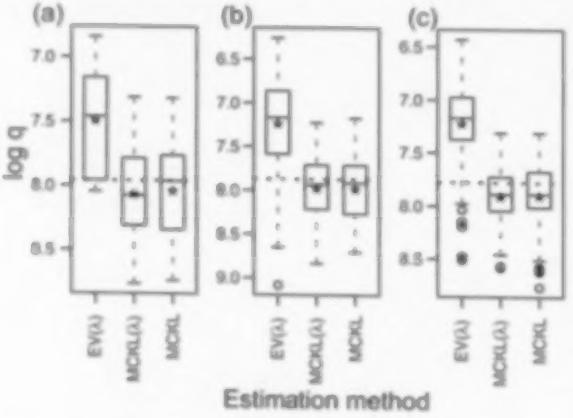
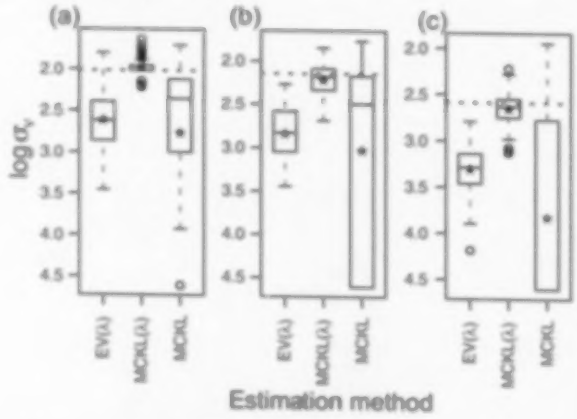


Fig. 5. Estimates of log(σ_v) for the hake (*Merluccius capensis* × *M. paradoxus*) model. Box plots of 100 point estimates of log(q) for each of three simulation parameters: (*a*) $\sigma_\varepsilon = 0.01$, (*b*) $\sigma_\varepsilon = 0.05$, (*c*) $\sigma_\varepsilon = 0.10$ (see text). EV, errors-in-variables; MCKL, Monte Carlo Kernel Likelihood. (λ) indicates that λ is assumed correctly known.



model investigated by Hilborn and Mangel (1997).

The model is

$$(21)\quad B(t+1) = \left[B(t) + rB(t)\left(1 - \frac{B(t)}{K}\right) - C(t)\right]\exp(v_t)$$

$$(22)\quad I(t) = qB(t)\exp(\varepsilon_t)$$

where $B(t)$, $C(t)$ and $I(t)$ are biomass, catch, and abundance index in year t, respectively; r is intrinsic growth rate; K is carrying capacity; q is fraction of biomass estimated by the survey; and v_t and ε_t are process and observation errors at time t, respectively, with means 0 and variances σ_v^2 and σ_ε^2, respectively. The parameters to be estimated are $\boldsymbol{\Theta} = (r, K, q, \sigma_v, \sigma_\varepsilon)$.

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Table 6. Mean, standard deviation (Std. Dev.), and root mean squared error (RMSE) for $\log(\hat{\sigma}_\varepsilon)$ for the hake (*Merluccius capensis* × *M. paradoxus*) example using errors-in-variables (EV; λ known) and Monte Carlo Kernel Likelihood (MCKL; λ known vs. unknown).

	Parameter used for simulation		
	$\Theta_{.01}$[a]	$\Theta_{.05}$[b]	$\Theta_{.10}$[c]
EV (λ known)			
Mean	−2.61	−2.83	−3.30
Std. Dev.	0.359	0.307	0.261
RMSE	0.698	0.757	0.760
MCKL (λ known)			
Mean	−1.97	−2.21	−2.65
Std. Dev.	0.0896	0.180	0.175
RMSE	0.0980	0.193	0.187
MCKL (λ unknown)			
Mean	−2.76	−3.02	−3.82
Std. Dev.	0.951	1.05	0.959
RMSE	1.21	1.37	1.56

[a] $\log(\sigma_\varepsilon) = -2.01$.
[b] $\log(\sigma_\varepsilon) = -2.14$.
[c] $\log(\sigma_\varepsilon) = -2.60$.

Table 7. Confidence interval (CI) performance of errors-in-variables (EV; λ known) and Monte Carlo Kernel Likelihood (MCKL; λ known vs. unknown) for the hake (*Merluccius capensis* × *M. paradoxus*) example.

	Parameters used for simulation		
Expected percentile	$\Theta_{.01}$	$\Theta_{.05}$	$\Theta_{.10}$
EV (λ known)			
0.05	1	1	1
0.1	1	1	1
0.25	1	1	1
0.5	1	1	1
MCKL (λ known)			
0.05	0.02	0.03	0.05
0.1	0.02	0.05	0.06
0.25	0.06	0.13	0.18
0.5	0.28	0.35	0.36
MCKL (λ unknown)			
0.05	0.06	0.09	0.06
0.1	0.12	0.14	0.11
0.25	0.27	0.30	0.31
0.5	0.41	0.48	0.49

Note: Row labels give nominal p value cutoff and table entries give actual number of simulations for which the true parameters were rejected using that cutoff.

To compare performance of MCKL and EV, we simulated 100 data sets using the real catch values and each of three sets of assumed parameter values, which were themselves estimated from the data using the MCKL method. For MCKL, minimum values for σ_ε and σ_v were set to 0.01. The three sets of parameters were (*i*) the MCKL maximum likelihood estimate (MLE) for all parameters, (*ii*) the MCKL MLE of (r, K, q, σ_v) with σ_ε fixed at 0.05, and (*iii*) the MCKL MLE of (r, K, q, σ_v) with σ_ε fixed at 0.10. The full MLE yielded $\hat{\sigma}_\varepsilon = 0.01$, the minimum value allowed because MCMC sampling is inefficient for smaller $\hat{\sigma}_\varepsilon$ values, so the latter two cases were used to include meaningful amounts of observation error in the simulations. This model-fitting problem showed a difficulty in distinguishing process and observation error similar to, but not as severe as, the linear autoregressive example. We label the cases as $\Theta_{.01}$, $\Theta_{.05}$ and $\Theta_{.10}$, with subscript indicating σ_ε.

For each of the three parameter scenarios, we simulated 100 sets of survey index time series using the true catch records. Then we estimated all parameters in three ways: EV with the correct λ, MCKL with λ assumed known and correct, and MCKL with λ unknown. As before, EV and MCKL with λ known differ only by using different likelihoods but share the same parameter space, while EV with λ unknown has a larger parameter space because it estimates both error variances.

Implementation details for the hake example

We used a standard Nelder–Mead (Press et al. 1992; *R* "optim" function) algorithm for all optimizations, with as many restarts as necessary to ensure convergence. For EV, we optimized from two sets of initial conditions: one based on a no-process-error (no-PE) state trajectory, the other based on a no-observation-error (no-OE) state trajectory, given the data and true parameters in both cases. Starting from each of these initial state trajectories, we optimized the parameters only, and then from those parameters we jointly optimized parameters and states. For the final estimates, we used the highest EV likelihood result from either the no-OE or no-PE initial conditions. Using the true parameters to estimate initial state conditions for the full maximization problem was cheating in the sense that we used the correct parameters to initialize the algorithm, but this allowed a high chance of locating the global EV maximum likelihood for proper comparison with MCKL, which was the primary goal here.

For MCKL, we used a nearly uninformative prior and a Metropolis–Hastings algorithm (Gilks et al. 1996; Robert and Casella 1999) to sample from $P[\log(\boldsymbol{\Theta}), \log(\mathbf{B}^T) | \mathbf{I}^T]$ (Appendix B). We kept every 100th sample to obtain $m = 40\,000$ points. For a single analysis, we would use a much larger sample, but here we used smaller samples to facilitate comparison of hundreds of replicated data series. For estimation of the three sets of parameters from which we simulated, we used samples of $m = 5 \times 10^6$.

In MCMC posterior sampling, the issue arises of how to treat the distribution of initial state values. One objective approach would be to use the stationary distribution of the model, but this is not generally analytically available. We used an approach that approximates the stationary distribution by including 10 years of unknown state variables before the first data, with the state at the first year (i.e., $t = -10$ if the data start at $t = 0$) distributed as $N(K, \sigma_\varepsilon^2)$, where K is the equilibrium value in the absence of harvest. This trick is limited to the case where the harvest data begin at the onset of substantial harvest, so that pre-data harvest can be assumed to be 0, which is approximately true for the hake case. This approach works because given any fixed initial state, the distribution of states will be the stationary distribution after a reasonable amount of time. Since we allow 10 years of stochastic state dynamics, unconstrained by observations, the resulting distribution for the tenth year (i.e., first year of data)

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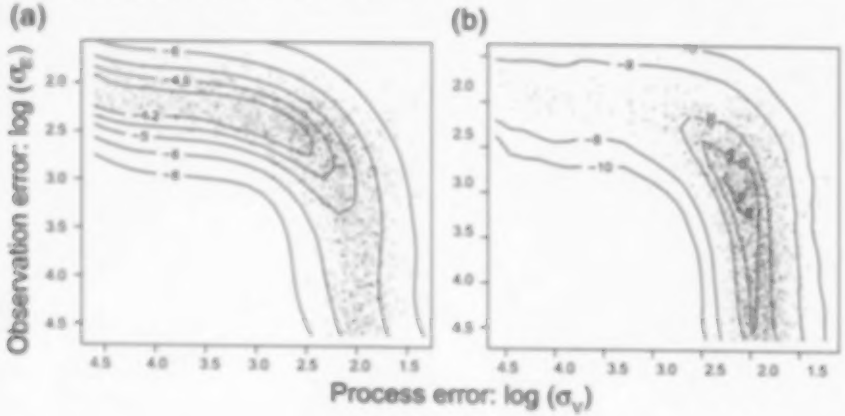
Fig. 6. Two example profile likelihood contours for $\log(\sigma_v)$ and $\log(\sigma_e)$ plotted over (thinned) marginal samples. Each figure shows 2000 scatter-plot points of a marginal posterior sample of $\log(\sigma_v)$ and $\log(\sigma_e)$ from simulated hake data. Contours show the profile likelihood (not the marginal density). For each point on a 25 × 25 grid of $\log(\sigma_v)$ and $\log(\sigma_e)$, the Monte Carlo Kernel Likelihood (MCKL) was maximized over the remaining three parameter dimensions, and the contours show approximate surfaces of equal maximum likelihood. Contour labels are log likelihoods plus an unknown constant. In (*a*), the MCKL maximum has the minimum allowed value for σ_v, but in (*b*), both σ_v and σ_e have intermediate values at the MCKL maximum. In both cases, the likelihood ridge over a wide range of σ_v and σ_e illustrates the difficulty of separating process and observation error.



will approximate the stationary distribution.

Maximization for MCKL also used the Nelder–Mead algorithm with multiple restarts. The algorithm was started at the mean posterior $\boldsymbol{\Theta}$ value, without any input based on true parameters. For MCKL with λ known, we used the full posterior sample but maximized under the constraint of known λ. For kernel density estimation, the kernel covariance was set to $\boldsymbol{\Sigma}_h = 0.1\hat{\boldsymbol{\Sigma}}_{\pi(\boldsymbol{\Theta}|I)}$, where $\hat{\boldsymbol{\Sigma}}_{\pi(\boldsymbol{\Theta}|I)}$ is the estimated covariance matrix of the posterior $\boldsymbol{\Theta}$ sample. The kernel density sometimes overlapped $\log(\sigma_v) < \log(0.01)$ or $\log(\sigma_e) < \log(0.01)$ (from which no points were sampled), so we renormalized the kernel density accordingly, allowing MCKL estimation of $\hat{\sigma}_v$ or $\hat{\sigma}_e$ near their minimum values.

Results for the hake example

Comparing EV and MCKL with λ known shows that EV is more biased, has higher RMSE, and has very inaccurate confidence regions, while MCKL with λ known has low bias and RMSE and usefully approximates confidence regions (Tables 4–7; Figs. 3–5). Comparing MCKL with λ known vs. unknown shows that estimates of ρ are similar, but when λ is unknown, separation of process and observation error is again difficult.

The difficulty of distinguishing process vs. observation error is illustrated by two examples of likelihood profiles (i.e. maximized over other parameters) in the $\log(\sigma_v) \times \log(\sigma_e)$ plane, plotted over a sample of the marginal posterior for illustration (Fig. 6). As illustrated in the first example, MCKL with unknown λ sometimes estimates an all-process-error or all-observation-error model. Nevertheless, the confidence regions for MCKL with λ unknown (reflected in p values of the true parameters) provide a useful estimate of uncertainty, even if the uncertainty is large. In many cases, the confidence region would include a span from all-process-error to all-observation-error parameters, reflecting the high level of uncertainty in these parameters.

Discussion

MCKL provides a method to calculate likelihoods from nonlinear state-space models with accuracy that increases as Monte Carlo sample size (computational effort) increases. Implementation of MCKL requires a method for obtaining a Bayesian posterior sample, generally by MCMC. Relevant MCMC samplers have been under active development in recent years. The additional steps for MCKL of calculating and maximizing a weighted kernel density estimate of a Bayesian posterior is relatively simple to implement.

The errors-in-variables method has long been recognized to be biased and requires difficult assumptions about the relative magnitudes of process and observation errors. Our study further explores these difficulties by comparison with state-space likelihoods that integrate over the distribution of unknown state trajectories. For both a linear and nonlinear model, integrated state-space likelihoods outperformed EV likelihoods. Integrated state-space likelihoods do not require assumptions about relative error magnitudes, although separation of process and observation error is an inherently noisy problem. Comparison of methods using other data and more complex models, such as age- or size-structured models, is an important topic for further investigation.

Fisheries scientists have increasingly turned to Bayesian methods in recent years. Our purpose here is not to compare and contrast Bayesian and classical methods but rather to separate the Bayesian vs. classical choice from model complexity, so that the best aspects of each approach are available. Bayesian methods have been highly associated with full incorporation of process and observation error for complex fisheries models largely because the boom in MCMC methods made posterior

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sampling feasible. With MCKL, classical likelihood analysis can also fully incorporate both types of error, so the pros and cons of each approach may be brought to bear on the same problems.

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Appendix A. EV estimation of autoregressive model.

Much of this appendix depends on assuming μ known. Since estimates of μ are well-behaved for both KF and EV methods, this assumption does not greatly confound the analytical results developed.

Derivation of l^{EV} for the AR (Example 1) case

We begin by deriving a relatively simple expression for the log of the EV likelihoods, $l^{EV} = \log(L^{EV})$. Throughout this appendix, we drop the superscript "T" notation, so $\boldsymbol{X} \equiv \boldsymbol{X}^T$ and $\boldsymbol{Y} \equiv \boldsymbol{Y}^T$, and we drop $\boldsymbol{\Theta}$ from the probability notation, so $P(\cdot) \equiv P(\cdot|\boldsymbol{\Theta})$.

The covariance matrix of $\boldsymbol{X}$ is $\boldsymbol{\Sigma}_{XX}$, where $(\boldsymbol{\Sigma}_{XX})_{ij} = \frac{\sigma_\varepsilon^2 \rho^{|i-j|}}{1-\rho^2}$. The covariance matrix of $\boldsymbol{Y}$ is $\boldsymbol{\Sigma}_{YY} = \boldsymbol{\Sigma}_{XX} + \sigma_\varepsilon^2 \mathbf{I}$, where $\mathbf{I}$ is the identity matrix.

For the linear Gaussian case, the EV likelihood is

$$L^{EV} = P[\boldsymbol{Y}|(\boldsymbol{X} = \hat{\boldsymbol{X}})]P(\boldsymbol{X} = \hat{\boldsymbol{X}}) \tag{A1}$$

where $\hat{\boldsymbol{X}}$ is the estimated state trajectory that maximizes L^{EV}. Because the distributions of $\boldsymbol{X}$ and $\boldsymbol{Y}$ are multivariate normal, the estimated states are equal to the mean of the conditional distribution of $\boldsymbol{X}$ given $\boldsymbol{Y}$ (cf. Schnute 1994):

$$\hat{\boldsymbol{X}} - \mu_{\boldsymbol{X}} = \boldsymbol{\Sigma}_{XY}\boldsymbol{\Sigma}_{YY}^{-1}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}}) \tag{A2}$$

where $\boldsymbol{\Sigma}_{XY}$ is the matrix of covariances between elements of $\boldsymbol{X}$ and $\boldsymbol{Y}$: $(\boldsymbol{\Sigma}_{XY})_{ij} = \mathrm{cov}(X_i, Y_j)$. Note that $\boldsymbol{\Sigma}_{XY} = \boldsymbol{\Sigma}_{YX}$.

The distribution of $\boldsymbol{Y} - \mu_{\boldsymbol{Y}}$ given $\boldsymbol{X}$ is

$$(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})|\boldsymbol{X} \sim N[\boldsymbol{X} - \mu_{\boldsymbol{X}}, \boldsymbol{\Sigma}_{YY} - \boldsymbol{\Sigma}_{YX}\boldsymbol{\Sigma}_{XX}^{-1}\boldsymbol{\Sigma}_{XY}] \tag{A3}$$

where $N(\boldsymbol{a}, \mathbf{b})$ denotes a normal distribution with mean vector $\boldsymbol{a}$ and covariance matrix $\mathbf{b}$. Note the covariance matrix of $(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})|\boldsymbol{X}$ reduces to $\sigma_\varepsilon^2\mathbf{I}$.

Using eqs. A2 and A3, the first term of l^{EV} is

$$\log[P[\boldsymbol{Y}|(\boldsymbol{X} = \hat{\boldsymbol{X}})]] = -n\log(\sigma_\varepsilon) - \frac{\sigma_\varepsilon^2(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})'(\boldsymbol{\Sigma}_{YY}^{-1})^2(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})}{2} \tag{A4}$$

Together with

$$\log P(\boldsymbol{X} = \hat{\boldsymbol{X}}) = -\frac{\log(|\boldsymbol{\Sigma}_{XX}|)}{2} - \frac{1}{2}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})'[\boldsymbol{\Sigma}_{YY}^{-1} - \sigma_\varepsilon^2(\boldsymbol{\Sigma}_{YY}^{-1})^2](\boldsymbol{Y} - \mu_{\boldsymbol{Y}}) \tag{A5}$$

we have for the log EV likelihood:

$$l^{EV} = -\frac{1}{2}\log(|\sigma_\varepsilon^2\boldsymbol{\Sigma}_{XX}|) - \frac{1}{2}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})'\boldsymbol{\Sigma}_{YY}^{-1}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}}) \tag{A6}$$

The log true likelihood, l^{KF}, is given simply by the normal distribution for $\boldsymbol{Y}$:

$$l^{KF} = -\frac{1}{2}\log(|\boldsymbol{\Sigma}_{YY}|) - \frac{1}{2}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})'\boldsymbol{\Sigma}_{YY}^{-1}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}}) \tag{A7}$$

which is equivalent to the KF.

Comparing eqs. A6 and A7, we see that l^{EV} mishandles the variances, since $|\boldsymbol{\Sigma}_{YY}| = |\boldsymbol{\Sigma}_{XX} + \sigma_\varepsilon^2\mathbf{I}|$, so

$$|\boldsymbol{\Sigma}_{YY}| \neq |\sigma_\varepsilon^2\boldsymbol{\Sigma}_{XX}| \tag{A8}$$

Note that $|\sigma_\varepsilon^2\boldsymbol{\Sigma}_{XX}|$ is $\sigma_\varepsilon^{2n}\sigma_\varepsilon^{2n}$ times a function of ρ, while $|\boldsymbol{\Sigma}_{YY}|$ is σ_ε^{2n} times a function of ρ and λ; therefore, l^{EV} includes twice as many variance terms in its normalization constant than does the true likelihood l^{KF}.

Asymptotic EV parameter estimates

We can obtain asymptotic expected parameter estimates for the EV method by maximizing the expected log likelihood. This is justified because as long as the random parts of l^{EV} converge to fixed values, we can take expectations of the EV likelihood and then maximize in the parameters, rather than maximizing in the parameters and then taking expectations. We do this with Matlab (The MathWorks Inc., Natick, Massachusetts) using a simplified expression for l^{EV}.

We simplify l^{EV} by simplifying $|\boldsymbol{\Sigma}_{XX}|$ and $E[(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})' \times \boldsymbol{\Sigma}_{YY}^{-1}(\boldsymbol{Y} - \mu_{\boldsymbol{Y}})]$. The notation $E[h(z)]$ denotes the expected value of $h(z)$. For the first part, we have

$$|\boldsymbol{\Sigma}_{XX}| = \frac{1}{|\boldsymbol{\Sigma}_{XX}^{-1}|} = \frac{\sigma_\varepsilon^{2n}}{1-\rho^2} \tag{A9}$$

To obtain this, consider the case $\sigma_\varepsilon^2 = 1$, so $\boldsymbol{\Sigma}_{XX}^{-1}$ has 1 in the upper left and lower right entries, $1 + \rho^2$ on all other diagonal entries, $-\rho$ above and below the diagonal, and 0 elsewhere. The determinant of this matrix is $1 - \rho^2$.

For the other piece of l^{EV}, we can use the following relations. If A is a multivariate, normal, random variable with covariance matrix $\boldsymbol{\Sigma}_{AA}$, we will be interested in the expected value of $A'\boldsymbol{\Sigma}_{BB}^{-1}A$, where $\boldsymbol{\Sigma}_{BB}$ is some covariance matrix. Write

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$\mathbf{\Sigma}_{BB}^{-1} = Q_B' \mathbf{\Lambda}_B^{-1/2} \mathbf{\Lambda}_B^{-1/2} \mathbf{Q}_B$. This is a standard factorization of covariance matrices (e.g., Johnson and Wichern 1992), where $\mathbf{Q}_B$ is an orthonormal rotation matrix, and $\mathbf{\Lambda}_B^{-1/2}$ is a diagonal matrix with entries that are the square roots of the eigenvalues. Now $Z = \mathbf{\Lambda}_B^{-1/2} \mathbf{Q}_B A$ is a random variable with covariance

$$\text{(A10)} \quad \mathbf{\Sigma}_{ZZ} = \mathbf{\Lambda}_B^{-1/2} Q_B' \mathbf{\Sigma}_{AA} \mathbf{Q}_B \mathbf{\Lambda}_B^{-1/2}$$

We want the expected value of $Z'Z$. This is given by

$$\begin{aligned} \text{(A11)} \quad E[Z'Z] &= \text{tr}(\mathbf{\Sigma}_{ZZ}) \\ &= \text{tr}(\mathbf{\Lambda}_B^{-1/2} Q_B' \mathbf{\Sigma}_{AA} \mathbf{Q}_B \mathbf{\Lambda}_B^{-1/2}) \\ &= \text{tr}(\mathbf{\Sigma}_{AA} \mathbf{\Sigma}_{BB}^{-1}) \end{aligned}$$

where for the last step tr denotes trace, and the relation $\text{tr}(CD) = \text{tr}(DC)$ has been used with $C = \mathbf{\Sigma}_{AA} \mathbf{Q}_B \mathbf{\Lambda}_B^{-1/2}$, $D = \mathbf{\Lambda}_B^{-1/2} Q_B'$.

Applied to the equation for l^{EV}, we get that if candidate parameters are given by $\hat{\mathbf{\Theta}}$, then

$$\text{(A12)} \quad E[(\mathbf{Y} - \mu)' \mathbf{\Sigma}_{\mathbf{YY},\hat{\mathbf{\Theta}}}^{-1} (\mathbf{Y} - \mu)] = \text{tr}(\mathbf{\Sigma}_{\mathbf{Ym}} \mathbf{\Sigma}_{\mathbf{YY},\hat{\mathbf{\Theta}}}^{-1})$$

Putting these pieces together gives

$$\begin{aligned} \text{(A13)} \quad E[l(\mathbf{\Theta})] = -\frac{1}{2}[n[\log(\hat{\sigma}_\varepsilon^2 \hat{\sigma}_v^2)] - \log(1 - \hat{\rho}^2) \\ - \text{tr}(\mathbf{\Sigma}_{\mathbf{YY}} \mathbf{\Sigma}_{\mathbf{YY},\hat{\mathbf{\Theta}}}^{-1})] \end{aligned}$$

The usefulness of this equation is that $\text{tr}(\mathbf{\Sigma}_{\mathbf{YY}} \mathbf{\Sigma}_{\mathbf{YY},\hat{\mathbf{\Theta}}}^{-1})$ can be easily computed and eq. A13 easily maximized computationally. Even with small values of n (e.g., 20), and hence computationally quick matrix sizes, the answer is only negligibly different than the answer with much larger matrix sizes. As a final note on the justification of this calculation, the trace term actually increases with n, but if one divides by n, the distribution of $\frac{1}{n}(\mathbf{Y} - \mu)' \mathbf{\Sigma}_{\mathbf{YY},\hat{\mathbf{\Theta}}}^{-1} (\mathbf{Y} - \mu)$ converges appropriately. Severini (2000) discusses this more generally and formally.

Asymptotic estimate of variance for true ρ

To obtain insight into EVs handling of variance estimation, consider the artificial case of known autocorrelation $\hat{\rho} = \rho$. Denote $\sigma^2 = \sigma_\varepsilon^2$ and define $\mathbf{\Sigma}^*$ by $\mathbf{\Sigma} = \sigma^2 \mathbf{\Sigma}^*$. Then

$$\text{(A14)} \quad l^{\text{EV}} = -2n \log(\sigma) - \frac{\log(|\mathbf{\Sigma}_{\mathbf{XX}}^*|)}{2} - \frac{(\mathbf{Y} - \mu_{\mathbf{Y}})(\mathbf{\Sigma}_{\mathbf{YY}}^*)^{-1}(\mathbf{Y} - \mu_{\mathbf{Y}})}{2\sigma^2}$$

Setting the derivative with respect to σ equal to zero gives the l^{EV} estimate:

$$\text{(A15)} \quad \hat{\sigma}^2 = \frac{(\mathbf{Y} - \mu_{\mathbf{Y}})(\mathbf{\Sigma}_{\mathbf{YY}}^*)^{-1}(\mathbf{Y} - \mu_{\mathbf{Y}})}{2n}$$

Even if we know the correct values of ρ and μ, so that $E[(\mathbf{Y} - \mu_{\mathbf{Y}})(\mathbf{\Sigma}_{\mathbf{YY}}^*)^{-1}(\mathbf{Y} - \mu_{\mathbf{Y}})] = n\sigma^2$, this estimator is biased by 50%:

$$\text{(A16)} \quad E(\hat{\sigma}^2) = \sigma^2/2$$

Although we will not generally know ρ, this result provides insight into the manner in which the EV method underestimates the true variance by using twice as many variances in the normalization constant compared with the true likelihood. Related results are discussed by Ludwig and Walters (1981), Schnute and Kronlund (2002), and references therein.

Asymptotic EV distribution of $\hat{\mathbf{X}}$

Given estimates $\hat{\rho}$ and $\hat{\sigma}^2$, we can calculate the distribution of $\hat{\mathbf{X}}$ from eq. A2. This is

$$\text{(A17)} \quad \hat{\mathbf{X}} - \mu_{\mathbf{X}} \sim N(0, \hat{\mathbf{\Sigma}}_{\mathbf{XX}} \hat{\mathbf{\Sigma}}_{\mathbf{YY}}^{-1} \mathbf{\Sigma}_{\mathbf{YY}} \hat{\mathbf{\Sigma}}_{\mathbf{YY}}^{-1} \hat{\mathbf{\Sigma}}_{\mathbf{XX}})$$

The covariances between $(\hat{\mathbf{X}} - \mu_{\mathbf{X}})$ and $(\mathbf{X} - \mu_{\mathbf{X}})$ are given by the matrix $\hat{\mathbf{\Sigma}}_{\mathbf{XY}} \hat{\mathbf{\Sigma}}_{\mathbf{YY}}^{-1} \mathbf{\Sigma}_{\mathbf{XX}}$

This matrix can be computed easily with mathematical software, and the appropriate terms can be used to calculate the slope of $\hat{X}_T$ on X_T:

$$\text{(A18)} \quad b = \frac{\text{cov}(\hat{X}_T, X_T)}{\text{var}(\hat{\mathbf{X}}_T)}$$

Hessians

In standard likelihood-based methods, one can obtain approximate CIs by using the fact that parameter estimates are asymptotically normally distributed. Some investigators (see text) use this result in the different context where parameters and states are included together and are hoped to be asymptotically normal in a similar way. Following the example of Richards et al. (1997), let $\mathbf{\Phi} = (\mathbf{\Theta}, X)$ (they use $\mathbf{\Theta}$ where we have used $\mathbf{\Phi}$). They propose that

$$\text{(A19)} \quad l(\mathbf{\Phi}) \approx l(\hat{\mathbf{\Phi}}) - \frac{1}{2}(\mathbf{\Phi} - \hat{\mathbf{\Phi}})' J(\hat{\mathbf{\Phi}})(\mathbf{\Phi} - \hat{\mathbf{\Phi}})$$

where

$$\text{(A20)} \quad J(\hat{\mathbf{\Phi}}) = -\frac{\partial^2 l}{\partial \mathbf{\Phi}^2}\Big|_{\mathbf{\Phi} = \hat{\mathbf{\Phi}}}$$

Under standard likelihood theory, this approximation is not valid, but would be valid if $\mathbf{\Phi}$ were replaced with $\mathbf{\Theta}$ (i.e., the parameters but not the states).

For the states, the elements of the Hessian are

$$\text{(A21)} \quad -\frac{\partial^2 l}{\partial \hat{\mathbf{X}}_i^2} = \frac{1}{\sigma_v^2}\left(\frac{1}{\gamma} + 1 + \rho^2\right), \quad i = 1, \ldots, T-1$$

$$\text{(A22)} \quad -\frac{\partial^2 l}{\partial \hat{\mathbf{X}}_i \partial \hat{\mathbf{X}}_{i+1}} = -\frac{\rho}{\sigma_v^2}, \quad i = 1, \ldots, T-1$$

$$\text{(A23)} \quad -\frac{\partial^2 l}{\partial \hat{\mathbf{X}}_T^2} = \frac{1}{\sigma_v^2}\left(\frac{1}{\gamma} + 1\right)$$

where $\gamma = \sigma_\varepsilon^2/\sigma_v^2$. Since these values are constant even as the amount of data increases, this is not the type of likelihood situation to which the usual asymptotic results apply. One could obtain analytic expressions for other elements of the Hessian, but for simplicity we calculated Hessians numerically using standard finite element methods.

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Appendix B. MCMC sampler for the hake model.

The MCMC algorithm sampled from the density $P(\boldsymbol{\Theta}, B_{-10}, \ldots, B_0, \ldots, B_T | I_0, \ldots, I_T) = P(\boldsymbol{\Theta}, \mathbf{B}|\mathbf{I})$, where the catch and index record starts at time 0. We used the log of all parameters, $\boldsymbol{\Theta} = (\log r, \log K, \log q, \log \sigma_v, \log \sigma_\varepsilon)$, as the sampling space. Biomass values for $t < 0$ are included in the model so that B_0 is distributed approximately as the stationary distribution of the model, which works because harvest levels were minor for $t < 0$ (see text). We define $C_t = 0$ for $t < 0$. For MCMC sampling, one only needs to be able to calculate the target density up to an unknown constant factor. In this case, we have $P(\boldsymbol{\Theta}, \mathbf{B}|\mathbf{I}) \propto P(\boldsymbol{\Theta}, \mathbf{B}, \mathbf{I})$, and the latter density was used to calculate MCMC acceptance probabilities. We write $\phi(x, \mu, \sigma)$ for the normal distribution density for value x given mean μ and standard deviation σ.

The total probability can be factored as

$$\text{(B1)}\quad P(\boldsymbol{\Theta}, \mathbf{B}, \mathbf{I}) = P_{\text{Prior}}(\boldsymbol{\Theta}) P(B_{-10}|\boldsymbol{\Theta}) \times \left[\prod_{t=-9}^{T} P(B_t|B_{t-1})\right]\left[\prod_{t=0}^{T} P(I_t|B_t)\right]$$

where

$$\text{(B2)}\quad P(B_t|B_{t-1}, \boldsymbol{\Theta}) = \phi[\log(B_t) - \log[B_{t-1} + r B_{t-1}(1 - \frac{B_{t-1}}{K}) - C_{t-1}], 0, \sigma_v], \quad t > -10$$

$$\text{(B3)}\quad P(B_{-10}|\boldsymbol{\Theta}) = \phi[\log(B_{-10}), \log(K), \sigma_v]$$

$$\text{(B4)}\quad P(I_t|B_t) = \phi[\log(I_t) - \log(q B_t), 0, \sigma_\varepsilon]$$

The prior $P_{\text{Prior}}(\boldsymbol{\Theta})$ was the product of independent priors for each parameter. For MCMC sampling, the priors need only be specified up to a constant. Priors for $\log(r)$, $\log(\sigma_v)$, and $\log(\sigma_\varepsilon)$ were each exponential with rate 0.0001 and minimum value log(0.01). For example

$$\text{(B5)}\quad P_{\text{Prior}}[\log(r)] = D \exp[-0.0001 \log(r)], \quad \log(r) > 0.01$$

where D is a normalizing constant that does not matter in the Metropolis–Hastings acceptance probability. The prior for $\log(K)$ was constant between 0 and log(8000), and that for $\log(q)$ was constant between $\log(10^{-5})$ and $\log(10^{-2})$.

The sampler iterated through a sequence of MCMC steps with one-dimensional proposals densities as well a joint [$\log(q)$, B] proposal. Here, prime indicates a proposal value, $N(\mu, \sigma)$ indicates a normal distribution, and $N_r(\mu, \sigma, \text{min or max})$ indicates a reflected normal distribution with mean μ, standard deviation σ, and minimum or maximum values specified. For example, if $(x'|x) \sim N_r(x, 1, 10)$ and $x < 10$, then $P(9.5|9) = \phi(0.5, 0, 1) + \phi(1.5, 0, 1)$ (i.e., a normal draw of 1.5 given $x = 9$ would be reflected at $x' = 10$ to give a proposal value of $x' = 9.5$). In practice, only the backwards-to-forwards ratio $P(x|x')/P(x'|x)$ enters the MCMC acceptance probability, and this is one for all of our proposal densities, including reflected normals. To obtain good mixing, we included for some parameters multiple proposal densities with different standard deviations to allow mixing at different scales for different parts of parameter space. One iteration of our sampler included Metropolis–Hastings steps with the following proposals:

(1) $[\log B_t' | \log(B_{t-1})] \sim N(\log B_{t-1}, 0.05)$

(2) $[\log(q')|\log(q)] \sim N_r[\log(q), 0.001, \log(0.01), \log(0.00001)]$

(3) $[\log(r')|\log(r)] \sim N_r[\log(r), 0.05, \log(0.01)]$

(4) $[\log(q')|\log(q)] \sim N_r[\log(q), 0.01, \log(0.01), \log(0.00001)]$

(5) $[\log(K')|\log(K)] \sim N_r[\log(K), 0.05, 0, \log(8000)]$

(6) $[\log(q')|\log(q)] \sim N_r[\log(q), 0.05, \log(0.01), \log(0.00001)]$

(7) $[\log(\sigma_v')|\log(\sigma_v)] \sim N_r[\log(\sigma_v), 0.05, \log(0.01)]$

(8) $[\log(\sigma_\varepsilon')|\log(\sigma_\varepsilon)] \sim N_r[\log(\sigma_\varepsilon), 0.05, \log(0.01)]$

(9) $[\log(q')|\log(q)] \sim N_r[\log(q), 0.1, \log(0.01), \log(0.00001)]$

and

(10) $[\log(q')|\log(q)] \sim N_r[\log(q), 0.05, \log(0.01), \log(0.00001)]$,

with $\log(B_t') = \log(B_t) + [\log(q) - \log(q')]$ for all t

Step 10 requires care because it samples in a transformed coordinate space, for which a Jacobian transformation of proposal probabilities is necessary, but in this case the transformation is linear and the Jacobian is constant, so the backwards-to-forwards ratio remains constant.

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Influence of physicochemical factors on the distribution and biomass of invasive mussels (*Dreissena polymorpha* and *Dreissena bugensis*) in the St. Lawrence River

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Abstract: Twenty sites along the St. Lawrence River were sampled to determine if the distribution and abundance of invasive mussels (zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*)) are explained by physicochemical variables. Calcium concentration, substrate size, and depth independently explained significant proportions of variation in biomass for both species. Zebra mussel populations occurred at calcium levels as low as 8 mg $Ca{\cdot}L^{-1}$, but quagga mussels were absent below 12 mg $Ca{\cdot}L^{-1}$, suggesting that they have higher calcium requirements. Both species increased in biomass with increasing substrate size but displayed contrasting patterns with depth. Using combinations of these environmental variables, we developed stepwise multiple regression models to predict zebra mussel biomass and quagga mussel biomass. The zebra mussel model included calcium concentration, substrate size, and depth ($r^2 = 0.36$, $P < 0.0001$), while the quagga mussel model included only substrate size and depth ($r^2 = 0.32$, $P < 0.0001$). These results suggest that dreissenid mussel abundance (and correlated impacts) will vary predictably across environmental gradients, but the same predictive model will not be accurate for both species.

Résumé : Nous avons échantillonné 20 sites le long du Saint-Laurent pour voir s'il est possible d'expliquer la répartition et l'abondance des moules envahissantes (la moule zébrée (*Dreissena polymorpha*) et la moule quagga (*Dreissena bugensis*)) d'après les variables physico-chimiques. Les concentrations de calcium, la taille du substrat et la profondeur expliquent de façon indépendante des portions importantes de la variation en biomasse des deux espèces. Les populations de moules zébrées se retrouvent à des concentrations de calcium aussi faibles que 8 mg $Ca{\cdot}L^{-1}$, mais les moules quagga sont absentes aux concentrations inférieures à 12 mg $Ca{\cdot}L^{-1}$, ce qui indique que ces dernières ont des besoins plus élevés en calcium. Les biomasses des deux espèces augmentent en fonction de la taille du substrat, mais elles suivent des patrons différents en fonction de la profondeur. En combinant ces variables du milieu, nous avons mis au point des modèles de régression multiple pas à pas pour prédire les biomasses des moules zébrées et des moules quagga. Le modèle des moules zébrées comprend les concentrations de calcium, la taille du substrat et la profondeur ($r^2 = 0{,}36$, $P < 0{,}0001$) et celui de la moule quagga contient seulement la taille du substrat et la profondeur ($r^2 = 0{,}32$, $P < 0{,}0001$). Ces résultats indiquent que l'abondance des bivalves dreissénidés et les impacts reliés varient de façon prédictive le long de gradients de l'environnement, mais qu'un même modèle prédictif ne peut être précis pour les deux espèces.

[Traduit par la Rédaction]

Introduction

A central goal of ecology is to explain patterns of species distribution and abundance. A predictive understanding of such patterns for invasive species, in particular, has both fundamental and applied importance because effective management of the damage caused by invasive species requires advanced knowledge of where the impact will be greatest (Parker et al. 1999). The impact of an invader is strongly correlated with its abundance (Parker et al. 1999; Ricciardi 2003); therefore, by relating an invader's abundance to environmental variables, predictive models can be developed that would help managers anticipate which habitats will be most affected by invasion. A valuable approach toward developing such models is the statistical synthesis of multisite data from invaded regions (Ramcharan et al. 1992; Mellina and Rasmussen 1994; MacIsaac et al. 2000).

Predictive models are rare for aquatic invasive species, including the zebra (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*), whose introduction into the Great Lakes in the late 1980s resulted in significant ecological and economic impacts (O'Neill 1997; Vanderploeg et al. 2002). Both species are native to rivers in the Black Sea region (Rosenberg and Ludyanskiy 1994) and were likely introduced via ballast water discharge from transoceanic vessels. The zebra mussel was first detected in Lake St. Clair in 1988 (Hebert et al. 1989) and has since spread rapidly into most river systems and hundreds of inland lakes in eastern North

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America (Ram and McMahon 1996). Quagga mussels were recorded only a year later in Lake Erie but have had a much more limited range expansion, thus far restricted mainly to the lower Great Lakes and the upper St. Lawrence River (Mills et al. 1993, 1996). By 1992, both species were present throughout the St. Lawrence River between Lake Ontario and Montreal, Quebec (Ricciardi et al. 1996). Initial colonization of the St. Lawrence River by dreissenid mussels is assumed to be the result of an eastward expansion through the lower Great Lakes and passive downstream dispersal from Lake Ontario (Griffiths et al. 1991; Mills et al. 1993). Today, the St. Lawrence River is still the only large North American river invaded by both species.

Although zebra mussels have an invasion history spanning nearly two decades in North America and extensive studies have been conducted on their life history and ecology, there exist few models to predict their distribution and abundance. Current models for zebra mussel populations in lakes and rivers are derived from their physiological and ecological requirements and include environmental factors such as calcium concentration (Ramcharan et al. 1992; Mellina and Rasmussen 1994), substrate size (Mellina and Rasmussen 1994), pH (Ramcharan et al. 1992), and total phosphorus (Wilson and Sarnelle 2002). In the St. Lawrence River, calcium concentration and substrate size have been found to have the strongest influence on zebra mussel occurrence and population density, respectively (Mellina and Rasmussen 1994). Zebra mussels require calcium for shell growth and osmoregulation (Vinogradov et al. 1993; McMahon 1996) and their populations are not viable below a threshold concentration estimated to be approximately 15 mg $Ca{\cdot}L^{-1}$ (Mellina and Rasmussen 1994). In addition, hard substrate is required for the settlement and survival of postveliger zebra mussels, at least during the early stages of invasion (Stanczykowska 1977; Lewandowski 1982). Indeed, zebra mussel density was correlated with substrate size in the St. Lawrence River in the early 1990s (Mellina and Rasmussen 1994). Zebra mussels preferentially settle and reach peak densities on hard substrates such as bedrock and boulders (Dermott and Munawar 1993). Over time, however, zebra mussels also colonize soft sediments, in some cases reaching densities comparable with those found on hard substrates (Berkman et al. 1998, 2000; Haltuch et al. 2000). Therefore, as time progresses, the strength of the relationship between zebra mussel density and substrate size could weaken. After more than a decade since the colonization of the St. Lawrence River, it is of interest to know whether the relationships observed by Mellina and Rasmussen (1994) still hold for zebra mussels and whether similar relationships exist for quagga mussels.

To date, no predictive models of abundance exist for quagga mussels and little is known about their environmental tolerances (McMahon 1996). Although the two species are closely related, they appear to have different patterns of distribution and abundance in relation to environmental factors. For example, zebra mussels preferentially colonize and dominate hard substrates in shallow waters (Dermott and Munawar 1993; Karatayev et al. 1998), whereas quagga mussels colonize a broader range of depths and substrates, often dominating colder, deeper waters and fine sediments (Dermott and Munawar 1993; Claxton et al. 1998; Mills et al. 1999). Therefore, different models may be required to predict the abundance of each species.

The objective of this study was to determine whether variation in dreissenid biomass in the upper St. Lawrence River (from the outflow of Lake Ontario to the Island of Montreal) is explained by calcium concentration, substrate size, and depth. We hypothesized that (*i*) calcium concentration would predict the occurrence but not the abundance of zebra and quagga mussels, (*ii*) substrate size would have no significant influence on zebra and quagga mussel biomass, and (*iii*) zebra mussel biomass would be greatest in shallow waters, while quagga mussel biomass would be greatest in deeper waters.

Methods

Study area

From mid-July to early September 2003, we sampled 20 sites located along the St. Lawrence River system between Prescott, Ontario, and Lac St. Louis at Lachine, Quebec (Fig. 1). Each site was sampled once over an approximate area of 100 m^2. This portion of the river was chosen because of the large variability in substrate quality and calcium concentration. A distinct calcium gradient occurs along the southwest shore of the island of Montréal, where the humic water of the Ottawa River mixes with the alkaline water of the main stem of the St. Lawrence River (Mellina and Rasmussen 1994). Ten of our study sites were established within this gradient to capture the variability in calcium (Fig. 1). Sampling depth across all sites ranged from 0.6 to 8.8 m (Table 1).

Sampling techniques

All sites were accessed from shore using self-contained underwater breathing apparatus (SCUBA), which allowed for accurate observation of bottom substrate. At each site, 10 replicate quadrats (enclosed by a 0.25-m^2 polyvinylchloride frame) were randomly placed on the bottom substrate (or on the canal walls at site 8; Table 1), with approximately 5–6 m between replicates. Owing to logistical constraints, fewer quadrats (five to nine) were sampled at three sites, and to capture local variability in depth and substrate, 11–16 quadrats were sampled at three other sites (Table 1). Water depth was measured at each quadrat location.

Substrate size

Substrate composition was determined in situ by visually estimating the percent areal coverage of each substrate type within the quadrat (listed in Table 2). The frame of the quadrat was premarked at specified intervals to ensure accurate estimations. If substrate composition was visibly homogenous, then mussels were collected from a smaller quadrat (0.0625 m^2) that was randomly placed within the 0.25-m^2 frame. Substrate size was classified a priori using a modification of the Wentworth classification (Hakanson and Jansson 1983). The average diameter (d) in millimetres for each substrate type was converted to the phi scale by transforming them to their negative log base 2 ($-\log_2 d$) values (Table 2). The phi value for each substrate type was multiplied by its percent areal cover and then summed to give the mean weighted particle size for each quadrat (following

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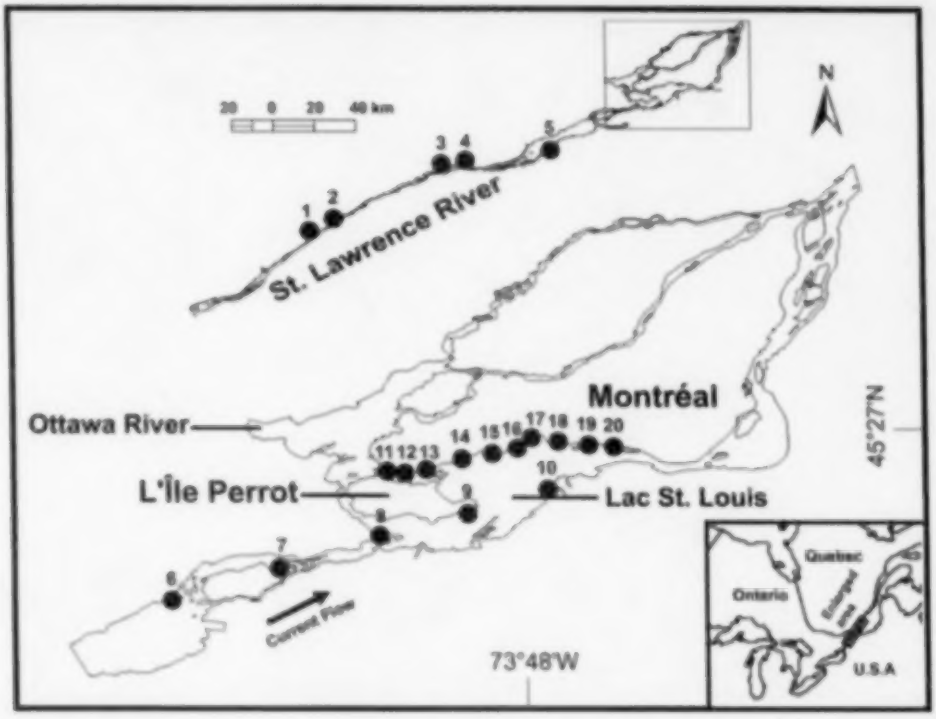
Fig. 1. Map of study sites along the St. Lawrence River. All sites are located between the outflow of Lake Ontario to the eastern tip of the island of Montréal (shown expanded).



Mellina and Rasmussen 1994). The ability to correctly classify substrate types in situ was verified by comparing visual estimations with measured diameters for 10 quadrats. Differences between these two methods did not exceed 5%. All measurements were performed by the same diver to minimize bias in assessing substrate characteristics.

Biomass

To estimate dreissenid biomass, we removed all visible mussels within the quadrat by hand and placed in marked, plastic collection bags. Quadrats were reexamined by two divers to ensure that no mussels were missed. Collection bags were stored in a cooler and transported back to the laboratory within 3 h of collection. Once in the laboratory, mussels were cleaned by hand, rinsed through a 1.0-mm-mesh sieve, and blotted dry to remove excess water. To minimize variation in wet mass during processing, we stored samples with water refrigerated at 4 °C until they were measured. This method successfully reduced the problem of shell gaping. We measured fresh mass (wet weight including shells) for zebra and quagga mussels to the nearest 0.1 g using an electronic balance. Biomass, rather than density, was the chosen measure of abundance because it is a better predictor of an invader's impact (Young et al. 1996; Wilson and Sarnelle 2002) and is less sensitive to errors caused by the incomplete removal of small, newly settled mussels from quadrats. We identified all mussels to species using a combination of morphological characters (Pathy and Mackie 1993; Claxton et al. 1997). The shell morphology of many quagga mussels collected at site 9 (Table 1) had external shell features that made identification difficult. The ventral surface was flattened with a ventrolateral ridge, as is occasionally observed in zebra mussels. Therefore, genetic analysis (mtDNA cytochrome *b* gene with polymerase chain reaction and autosequencing; Stepien et al. 2003) was used to confirm the species identity of these mussels (C.A. Stepien, Lake Erie Center, University of Toledo, 6200 Bayshore Road, Oregon, OH 43618, USA, personal communication).

Calcium concentration

On each sampling occasion, we took two water samples in 1.0-L plastic bottles. From these samples, calcium concentration (milligrams Ca^{2+} per litre, converted from milligrams $CaCO_3$ per litre) was measured with a LaMotte hardness model PHT-CM-DR-LT kit (LaMotte Company, Chestertown, Maryland) and the mean of the two sample measurements was used in our statistical analysis. For half of the sites, we were able to compare our values with measurements taken during the summer of 2002 and 2003, and they were found to be similar (±2 mg $Ca{\cdot}L^{-1}$).

Statistical analyses

All statistical analyses were performed using the SAS System for Windows V8 (SAS Institute Inc., Cary, North Carolina). To avoid violating assumptions underlying regression analysis and to directly test for the effect of local environmental variables on variation in biomass, we removed all quadrats with zero mussel biomass from the analysis. To test the putative calcium threshold of 15 mg $Ca{\cdot}L^{-1}$ (Mellina and Rasmussen 1994), we used χ^2 analysis to examine whether

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Table 1. Dreissenid biomass and physicochemical variables for 20 sites sampled along the St. Lawrence River (refer to Fig. 1 for site locations).

Site	No. of quadrats	Species	Biomass ($g \cdot m^{-2}$)	Substrate size (phi scale)	Depth (m)	Ca^{2+} ($mg \cdot L^{-1}$)
1	11	Z	86.1 (30.5)	–0.04 (2.0)	5.8 (0.8)	28.8 (0.2)
		Q*	622.8 (167.3)			
2	10	Z*	600.6 (143.4)	–0.84 (0.5)	2.7 (0.1)	28.4 (0.1)
		Q	177.6 (68.8)			
3	10	Z	78.6 (31.0)	–5.46 (1.4)	2.2 (0.4)	25.6 (0.1)
		Q	90.4 (36.0)			
4	10	Z*	370.5 (91.1)	–2.77 (1.4)	1.4 (0.1)	28.8 (0.3)
		Q	32.5 (7.3)			
5	10	Z	260.6 (73.6)	0.91(1.5)	3.0 (0.1)	26.0 (0.2)
		Q	33.8 (15.6)			
6	10	Z	1969.0 (423.7)	–7.92 (0.4)	2.1 (0.2)	30.0 (0.7)
		Q	1384.9 (431.0)			
7	10	Z	1588.1 (293.1)	–8.71 (0.2)	1.9 (0.4)	22.4 (0.1)
		Q	896.2 (243.8)			
8	16	Z	479.9 (118.2)	–4.04 (2.1)	3.5 (0.3)	26.0 (2.2)
		Q*	1722.6 (309.6)			
9	10	Z	690.7 (155.1)	–9.13 (0.1)	2.0 (0.2)	26.4 (1.2)
		Q	788.4 (198.6)			
10	10	Z*	1435.8 (200.5)	–6.56 (0.7)	1.5 (0.2)	24.8 (0.6)
		Q	153.4 (55.7)			
11*	10	Z	0.0	–6.09 (0.8)	1.5 (0.2)	7.6 (0.5)
		Q	0.0			
12	10	Z	5.4 (4.0)	8.81 (0.2)	1.3 (0.1)	10.0 (0.5)
		Q	0.0			
13	15	Z*	12.6 (4.2)	7.62 (0.3)	1.3 (0.1)	9.6 (0.1)
		Q	0.0			
14	9	Z	3.4 (2.0)	–6.52 (0.2)	1.3 (0.1)	8.0 (0.4)
		Q	0.0			
15	10	Z*	266.8 (91.2)	3.18 (1.4)	1.5 (0.2)	12.4 (0.2)
		Q	5.6 (3.0)			
16	5	Z	487.5 (118.8)	4.10 (2.4)	1.7 (0.1)	14.4 (0.4)
		Q	12.5 (10.9)			
17	10	Z*	457.0 (82.6)	7.52 (0.5)	1.4 (0.1)	23.8 (0.1)
		Q	21.4 (13.7)			
18	8	Z*	758.1 (412.3)	–3.08 (1.9)	1.5 (0.1)	13.2 (0.1)
		Q	44.2 (27.4)			
19	10	Z*	168.4 (51.3)	–7.72 (0.5)	1.3 (0.1)	18.4 (0.4)
		Q	7.1 (5.6)			
20	10	Z*	2183.4 (536.1)	–8.55 (0.4)	1.7 (0.1)	23.6 (0.6)
		Q	28.9 (12.2)			

Note: Values reported are the site means with standard error in parentheses. An asterisk indicates species with significantly higher average biomass per one square metre ($P < 0.05$) following sequential Bonferroni correction at $\alpha = 0.05$. Z, zebra mussels (*Dreissena polymorpha*); Q, quagga mussels (*Dreissena bugensis*).

*No statistical test done; biomass = 0 for both zebra mussels and quagga mussels.

the frequency of zero biomass values for both zebra and quagga mussels was independent of being above or below this threshold. In all regression analyses, zebra and quagga mussel biomass values were log transformed to reduce the influence of any extreme values and to stabilize variance (Downing 1979). Separate sequential Bonferroni corrections ($\alpha = 0.05$) were used to control the overall error rate of multiple tests (Rice 1989).

Univariate models were used to determine the variation in biomass across all quadrats that could be accounted for by individual predictor variables. Both linear and curvilinear regressions were tested. All significant regression slopes (both zebra and quagga mussels) were tested for homogeneity (Zar 1999). Finally, stepwise multiple regression models were developed to determine the combinations of environmental variables that explained the most variation in zebra mussel and quagga mussel biomass across quadrats.

Results

Mean zebra mussel and quagga mussel biomass varied broadly across sites (from 0 to 2183.4 ± 536.1 $g \cdot m^{-2}$ and

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Table 2. Substrate size classification and calculated phi values (modified from the Wentworth scale and Hakanson and Jansson (1983)).

Substrate type	Average diameter (mm)	Phi value
Clay–mud	<0.002	9.000
Silt	0.010	6.500
Sand	0.250	2.000
Gravel	13.250	–3.728
Cobble	73.000	–6.190
Rock	210.500	–7.718
Boulder	400.000	–8.644
Bedrock or artificial substrate	>700.000	–9.451

from 0 to 1722.6 ± 309.6 g·m^{-2}, respectively) (Table 1). Zebra mussels were found at 19 of the 20 sites and had a significantly higher biomass than quagga mussels at nine of these sites (*t* tests, $P < 0.005$, significant after sequential Bonferroni correction) (Table 1). Quagga mussels were present at 16 of the 20 sites but had a significantly higher biomass than zebra mussels at only two sites: site 8 (Soulanges Canal) and site 1 (Prescott, Ontario) (*t* tests, $P < 0.006$, significant after sequential Bonferroni correction) (Table 1). Only one site, located at the outflow of the Ottawa River (site 11), was devoid of both zebra and quagga mussels (Table 1; Fig. 1).

Calcium concentration, substrate size, and depth independently explained significant proportions of variation in biomass for both species (Table 3). Calcium concentration alone explained 21% and 10% of the variability in zebra mussel and quagga mussel biomass, respectively. Zebra mussel biomass increased with calcium concentrations up to 25 mg·L^{-1} but was reduced at sites with calcium concentrations above 25 mg Ca·L^{-1} (Fig. 2*a*). We found a strong log-linear relationship for zebra mussel biomass versus calcium below the putative threshold of 15 mg Ca·L^{-1} ($r^2 = 0.51$, $P < 0.0001$), but no relationship was found above 15 mg Ca·L^{-1} ($P = 0.21$).

Quagga mussel biomass was related to calcium concentration by a simple linear model ($r^2 = 0.10$, $P = 0.0005$) (Fig. 2*b*). For both zebra mussels and quagga mussels, χ^2 analysis revealed a higher than expected frequency of zero biomass values located below 15 mg Ca·L^{-1} than above ($\chi^2 = 48.39$, df = 1, $P = 0.001$, and $\chi^2 = 91.51$, df = 1, $P = 0.001$, respectively). Quagga mussels did not occur at sites below 12 mg Ca·L^{-1}, and zebra mussels did not occur at sites below 8 mg Ca·L^{-1}.

Both zebra and quagga mussel biomass declined with decreasing substrate size (i.e., increasing phi value), which explained 20% and 11% of the variation in their respective biomasses (Fig. 3). Nonetheless, high levels of biomass for both species were still observed in quadrats dominated by silt–mud (Fig. 3). The relationship between biomass and substrate size was not significantly different between species ($b_{D.\ polymorpha} = -0.05$, $b_{D.\ bugensis} = -0.05$; *t* test, $P > 0.50$ and $\text{intercept}_{D.\ polymorpha} = 2.27$, $\text{intercept}_{D.\ bugensis} = 1.96$; *t* test, $P > 0.50$).

Regression slopes for biomass against depth were significantly different for the two species ($b_{D.\ polymorpha} = -0.12$ and $b_{D.\ bugensis} = 0.16$; *t* test, $P < 0.05$). Quagga mussel biomass increased with depth (Fig. 4*a*), whereas zebra mussel biomass decreased with depth (Fig. 4*b*). Depth explained only a small proportion (≤10%) of the variation in biomass for either species.

Stepwise multiple regression showed that substrate size, calcium concentration, and depth collectively explained 36% of the variation in zebra mussel biomass ($P < 0.0001$) (Table 4). Only substrate size and depth significantly explained variation in quagga mussel biomass ($r^2 = 0.32$, $P < 0.0001$) (Table 4). Results for both models were robust to the order in which variables were entered.

Discussion

Few studies have attempted to develop empirical models of zebra mussel occurrence or abundance in river systems. No such attempt has been made for quagga mussels. To date, models of zebra mussel occurrence have been constructed from limnological variables (e.g., water hardness), while abundance models have included some measure of substrate availability. Here, we use an approach that combines these variables. Our analysis shows that about one third of the variation in dreissenid biomass in the upper St. Lawrence River is explained by calcium concentration, substrate size, and depth.

Calcium concentration as a predictor

Zebra mussels require calcium for shell production, growth, and survival. Previous studies have shown that calcium concentration limits the occurrence of zebra mussels but has proven to be a poor predictor of their numerical density (Stanczykowska 1964; Ramcharan et al. 1992; Mellina and Rasmussen 1994). In regards to occurrence, laboratory studies have found the minimum calcium threshold for normal development and long-term survival of zebra mussels to range from 8.5 to 12 mg Ca·L^{-1} (Sprung 1987; Vinogradov et al. 1993; Hincks and Mackie 1997). Meanwhile, field studies in North America to date have found no zebra mussels below 15 mg Ca·L^{-1} (Mellina and Rasmussen 1994; Strayer et al. 1996; Allen and Ramcharan 2001). Our study lends equivocal support for this threshold. More empty (zero biomass) quadrats occurred at sites with calcium levels below the 15 mg Ca·L^{-1} threshold than expected by chance. However, even at calcium levels below this threshold, quadrats were found containing zebra mussels, including some with high biomass (up to 3225.6 g·m^{-2} at 13 mg Ca·L^{-1}, site 18). It was not until concentrations below 10 mg Ca·L^{-1} that zebra mussel biomass declined precipitously and until 7.6 mg Ca·L^{-1} that mussels were absent. This suggests that the threshold for occurrence may be between 8 and 10 mg Ca·L^{-1}, which is consistent with the findings of Hincks and Mackie (1997), who reported negative growth of juvenile zebra mussels at calcium levels below 8.5 mg Ca·L^{-1}. Whether zebra mussels are able to reproduce in such low calcium concentrations remains to be tested, but it indicates that at least settlement and growth are possible. This apparent lower calcium threshold for zebra mussels may be a reflection of their adaptive ability. Zebra mussels are a phenotypically plastic and fecund species with a high potential for rapid adaptation to new environments (Mills et al.

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Table 3. Univariate linear regressions of three physicochemical variables with log-transformed biomass for zebra mussels (*Dreissena polymorpha*) (n = 162) and quagga mussels (*Dreissena bugensis*) (n = 123).

	Zebra mussels					Quagga mussels			
Variable	b (SE)	b_1 (SE)	Intercept (SE)	r^2	P	b (SE)	Intercept (SE)	r^2	P
$[Ca^{2+}]$ (mg·L^{-1})	0.29 (0.06)	–0.01 (0.002)	–0.64 (0.56)	0.21	<0.0001	0.06 (0.02)	0.60 (0.44)	0.10	0.0005
Substrate size (mm)	–0.05 (0.01)	—	2.27 (0.06)	0.20	<0.0001	–0.05 (0.01)	1.96 (0.09)	0.11	<0.0001
Depth (m)	–0.12 (0.04)	—	2.69 (0.10)	0.05	0.0030	0.16 (0.04)	1.79 (0.13)	0.10	0.0005

Note: Quadrats with zero zebra or quagga mussel biomass were excluded from their respective analyses. Regressions shown are significant after sequential Bonferroni correction (α = 0.05). b is slope and b_1 is slope for the second-degree term (calcium^2) for the quadratic linear regression model.

Fig. 2. Relationship between (*a*) zebra mussel (*Dreissena polymorpha*) and (*b*) quagga mussel (*Dreissena bugensis*) biomass (log transformed) and calcium concentration across quadrats. Quadrats with zero biomass for zebra mussels or quagga mussels were excluded from their respective analyses. The regression equations for zebra and quagga mussel biomass, respectively, are log biomass = –0.64 + 0.29·calcium – 0.01·calcium^2 (r^2 = 0.21, P < 0.0001, n = 162) and log biomass = 0.60 + 0.06·calcium (r^2 = 0.10, P = 0.0005, n = 123). The dotted line (*i*) represents the threshold calcium concentration of 15 mg·L^{-1} below which no zebra mussels were found in a previous study in the St. Lawrence River (Mellina and Rasmussen 1994).

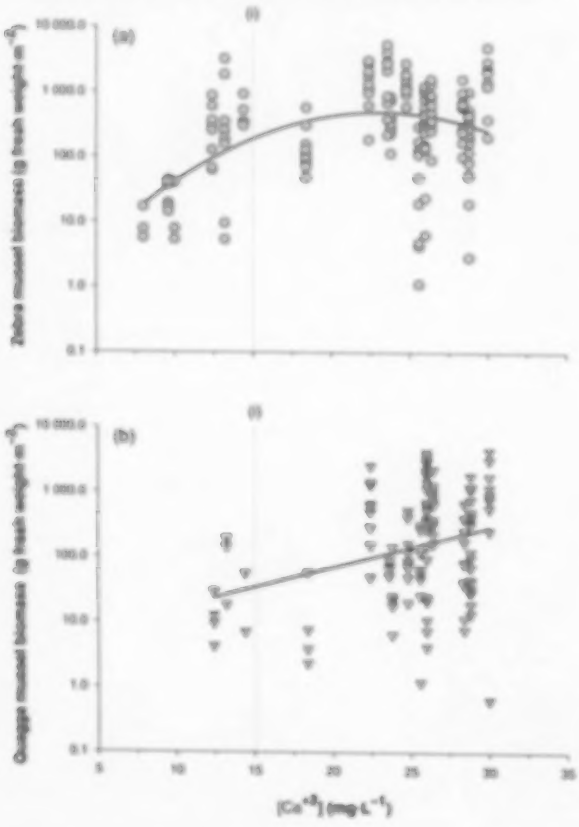
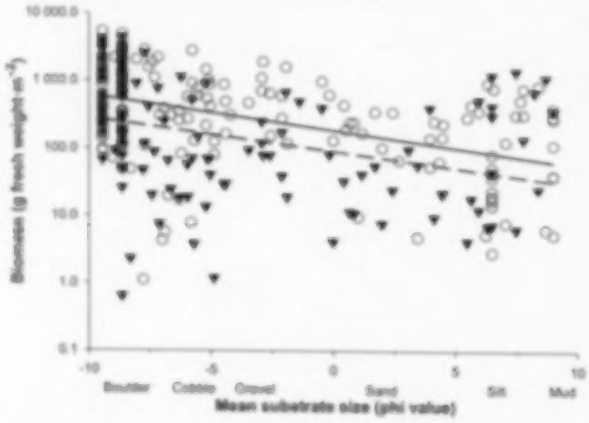


Fig. 3. Relationship between zebra mussel (*Dreissena polymorpha*) (circles, solid line) and quagga mussel (*Dreissena bugensis*) (triangles, broken line) biomass (log transformed) and substrate size expressed as mean weighted phi value (–log$_2$, mm) per quadrat. Quadrats with zero biomass for zebra mussels or quagga mussels were excluded from their respective analyses. The regression equations for zebra and quagga mussel biomass, respectively, are log biomass = 2.27 – 0.05·substrate size (r^2 = 0.20, P < 0.0001, n = 162) and log biomass = 1.96 – 0.05·substrate size (r^2 = 0.11, P = 0.0001, n = 123).



1996). Therefore, while the presence of a dense zebra mussel population below 12 mg Ca·L^{-1} is highly unlikely, predictions that use this critical value to map the potential spread of zebra mussels (e.g., Neary and Leach 1992) may ultimately underestimate zebra mussel distribution. In summary, zebra mussel biomass in the St. Lawrence River increased linearly with increasing calcium concentrations below 15 mg Ca·L^{-1}, peaked between 23 and 25 mg Ca·L^{-1}, and decreased thereafter, suggesting a unimodal response to calcium concentration. A negative effect of high calcium levels was also observed by Hincks and Mackie (1997), who found that adult mortality increased above 25 mg Ca·L^{-1} and maximum juvenile growth rates decreased above 32 mg Ca·L^{-1}.

In contrast with the detailed studies on the effect of calcium on zebra mussels, very little is known of the calcium requirements and tolerance limits of quagga mussels (McMahon 1996). We compared the frequency of empty (zero biomass) quagga mussel quadrats at sites above and below the reported zebra mussel threshold of 15 mg Ca·L^{-1}. The observed frequency of zero biomass values at concentrations below 15 mg Ca·L^{-1} was significantly higher than expected by chance. Quagga mussels were not found below 12 mg Ca·L^{-1}, suggesting that they have higher calcium requirements than zebra mussels. Furthermore, unlike zebra

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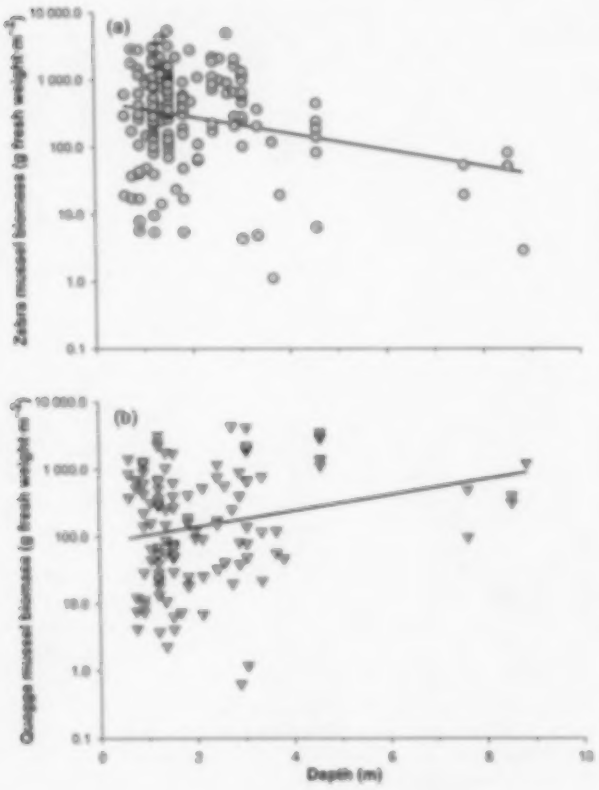
Table 4. Stepwise multiple regression models developed to predict log-transformed biomass for zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*) across quadrats from three independent physicochemical predictor variables.

	Equation	r^2	P	n
Zebra mussels	log biomass = 1.62 – 0.03·substrate size (0.01)** + 0.05·calcium (0.01)** – 0.19·depth (0.04)**	0.36	<0.0001	162
Quagga mussels	log biomass = 1.25 – 0.08·substrate size (0.01)** + 0.26·depth (0.04)**	0.32	<0.0001	123

Note: Numbers in parentheses are standard error of estimated coefficients and n is sample size. Quadrats with zero zebra or quagga mussel biomass were excluded from their respective analyses. Variables were retained in the model if significant at $P = 0.05$. **, $P < 0.0001$.

Fig. 4. Relationship between (*a*) zebra mussel (*Dreissena polymorpha*) and (*b*) quagga mussel (*Dreissena bugensis*) biomass (log transformed) and depth across quadrats. Quadrats with zero biomass for zebra mussels or quagga mussels were excluded from their respective analyses. The regression equations for zebra and quagga mussel biomass, respectively, are log biomass = 2.69 – 0.12·depth ($r^2 = 0.05$, $P = 0.003$, $n = 162$) and log biomass = 1.79 + 0.16·depth ($r^2 = 0.10$, $P = 0.0005$, $n = 123$).



mussels, quagga mussel biomass did not decline at high calcium concentrations. These results agree with a recent study of dreissenid mussels in Russian rivers, which concluded that quagga mussels tolerate higher calcium levels than zebra mussels (Zhulidov et al. 2004).

One potential criticism is that our measurements of calcium concentration are point measurements that do not incorporate seasonal and annual fluctuations. However, seasonal fluctuations in calcium concentrations tend to be limited in hardwater systems like the St. Lawrence River (Wetzel 1983). Moreover, our calcium concentrations are similar to those previously found in the St. Lawrence River (Mellina and Rasmussen 1994) as well as to measurements taken from the previous 2 years at 10 of our sites (A. Ricciardi, unpublished data).

Substrate size as a predictor

Substrate size is an ecological determinant of zebra mussel density (Mellina and Rasmussen 1994). The shell morphology of adult zebra mussels allows them to attach firmly to solid surfaces (Morton 1993), and adults preferentially colonize hard substrata in rivers and lakes (Dermott and Munawar 1993; Mellina and Rasmussen 1994; Karatayev et al. 1998). Following their initial introduction into the Great Lakes, zebra mussel populations grew explosively, reaching densities of 300 000 individuals·m^{-2} on rocky substrates (Griffiths et al. 1991; Leach 1993). Similarly, in the early stages of invasion of the St. Lawrence River, the highest zebra mussel densities occurred on hard substrates and substrate size explained 38% of the variation in mussel density (Mellina and Rasmussen 1994). However, the importance of primary hard substrate may diminish over time as zebra mussels begin to form byssally attached mats that expand from hard-substrate nuclei onto adjacent soft substrates such as sand, mud, and gravel (Hunter and Bailey 1992; Berkman et al. 2000). Zebra mussel occurrence and abundance on soft substrates within the Great Lakes has progressively increased, even without the assistance of hard nuclei in some areas (Berkman et al. 2000; Haltuch et al. 2000). This being the case, we expected that substrate size would not remain an important predictor of zebra mussel abundance in the St. Lawrence River 10 years after invasion. Yet, in our study, substrate size accounted for 20% of the variation in zebra mussel biomass (which is highly correlated with mussel density; $r^2 = 0.91$, $P < 0.0001$). However, we observed frequent use of soft substrates by zebra mussels and biomasses as high as 900 g·m^{-2} on sand and silt sediments.

Unlike zebra mussels, quagga mussels are thought to prefer soft substrata (Dermott and Munawar 1993; Dermott and Kerec 1997), but the relationship between substrate size and quagga mussel biomass has not been previously tested. In the Dneiper River drainage basin of the Ukraine, quagga mussels displaced zebra mussels from the rocky littoral zone within 4–12 years and similar trends are emerging in the Great Lakes (Mitchell et al. 1996; Mills et al. 1999) and St. Lawrence River (Ricciardi and Whoriskey 2004). This species shift suggests that, given enough time, the quagga mussel can eventually dominate a broad range of substrates (Mills et al. 1999; Stoeckmann 2003). Considering this pat-

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tern of invasion and that quagga mussels have been present in the St. Lawrence River system since at least 1992, it was surprising to find a relationship between substrate size and quagga mussel biomass. Moreover, despite suggested differences in substrate affinity, zebra and quagga mussels responded similarly to variation in substrate size.

Native unionids and macrophytes are also common substrates for dreissenid colonization (Lewandowski 1976; Ricciardi et al. 1996; Diggins et al. 2004). The presence of unionid mussels was a significant predictor of zebra mussel density in the multiple regression models of Mellina and Rasmussen (1994). However, unionids in the St. Lawrence River have declined substantially because of zebra mussel fouling (Ricciardi et al. 1996) and are now scarce in most areas of the river. While neither macrophytes nor unionid shells were abundant at any of our study sites, they represent potentially important substrates that could be incorporated in future models for other river systems.

Depth as a predictor

Zebra mussels generally reach their highest densities in shallow water, whereas quagga mussels tend to dominate deeper waters (Dermott and Munawar 1993; Mills et al. 1993). In Lake Erie, zebra and quagga mussels coexist at depths from 8 to 110 m, but only quagga mussels are present at depths greater than 110 m (Mills et al. 1999). However, it was not known whether a similar pattern would occur in the upper St. Lawrence River, where depth outside the shipping channel rarely exceeds 10 m. In our study, we found that zebra mussel biomass decreased with depth, while quagga mussel biomass increased, even over a narrow depth range (<9 m). Depth zonation of the two dreissenids has been suggested to be related to differences in temperature and silt tolerances as well as substrate preferences (Domm et al. 1993; Mitchell et al. 1996). However, temperature did not differ over the depth range of our study, and the two species have overlapping substrate preferences (and there is no simple relationship between substrate size and depth at littoral sites in the St. Lawrence River). This pattern may reflect differences in tolerance to natural disturbance and periodic aerial exposure (Mitchell et al. 1996; Ricciardi and Whoriskey 2004). Quagga mussels have a lower desiccation tolerance (Ricciardi et al. 1995) and their rounded shells might not be able to adhere as tightly to hard substrates (Pathy and Mackie 1993; Mills et al. 1996) and could thus be dislodged by wave action or ice scour.

Other factors affecting abundance and distribution

Some of the residual variation in dreissenid biomass might be explained by turbidity, which varies spatially and temporally in large heterogeneous river systems. Increased turbidity can dilute seston quality by increasing the fraction of suspended inorganic material (e.g., silt, clay). Seston quality affects bivalve respiration rate (Stoeckmann 2003), which is correlated with growth rate (Madon et al. 1998; Stoeckmann and Garton 2001). When seston quality is poor, quagga mussels have a lower respiration rate (Summers et al. 1996) and are more efficient than zebra mussels at acquiring food (Stoeckmann 2003). Zebra mussels have difficulty processing high levels of suspended inorganic particles (Madon et al. 1998). Therefore, under turbid conditions, quagga mussels should be less energetically stressed and attain higher biomasses than zebra mussels.

Another factor is larval supply. Riverine populations of dreissenids are considered to be dependent on larval recruitment from upstream lentic populations (Thorp et al. 2002). The dominant water source along the north shore of Montreal is from the Ottawa River, which has scarce zebra mussel populations and no known quagga mussel populations. Although mixing of water from both rivers occurs along the north shore (thus creating the calcium gradient), and complex current flows may allow for limited upstream dispersal (Thorp et al. 2002), a quantitative measure of larval supply could help tease apart the abiotic (calcium) and biotic (larval supply) effects on dreissenid occurrence.

Stepwise regression models

Despite that zebra and quagga mussels are closely related and functionally similar, the range of habitats that they may invade are significantly different. The multiple regression models that we developed for these two species differed in three important ways. First, while all three factors were retained in the zebra mussel model, only depth and substrate size were kept in the quagga mussel model. Second, the most important predictor of zebra mussel biomass (substrate size) differed from the most important predictor of quagga mussel biomass (depth). Third, while zebra mussel biomass decreased with increasing water depth, quagga mussel biomass increased. These findings suggest that one general model is not sufficient for predicting both zebra and quagga mussel biomass.

In summary, we have identified some important environmental factors that explain the distribution and biomass of zebra and quagga mussels. We found that calcium concentration sets a minimum threshold level for presence of both species but that quagga mussels appear to have a higher calcium requirement. The occurrence of zebra mussels at calcium concentrations as low as 8 mg $Ca{\cdot}L^{-1}$ indicates that they are able to colonize a broader range of habitats than previously expected. More than a decade after colonization, both zebra and quagga mussel biomass peaked on hard substrates, indicating that the two species have overlapping substrate preferences, contrary to conventional descriptions. However, substrate size is not as important in explaining zebra mussel abundance as it was during the early stages of invasion in the St. Lawrence River, which suggests that the accuracy of predictive models may change over time. In spite of the narrow depth range of this study, contrasting patterns of biomass for both species occurred with depth, with a trend toward quagga mussel domination in deeper waters. Finally, we derived a predictive model of biomass for each species from different combinations of local environmental variables, which demonstrated that models for one species are not appropriate for the other species. These variables provide a useful basis for predicting future zebra mussel and quagga mussel distribution and biomass in other invaded river systems.

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Methyl mercury concentrations in macroinvertebrates and fish from burned and undisturbed lakes on the Boreal Plain

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Abstract: Methyl mercury (MeHg) concentrations in macroinvertebrates and fish were compared among five lakes in burned catchments and five reference lakes on the western Canadian Boreal Plain to determine the influence of forest fire on MeHg bioaccumulation. Two years after fire, MeHg concentrations in five of six aquatic taxa were similar in burned and reference lakes. Among a larger set of 12 lakes, MeHg concentrations in biota were negatively correlated with lake water pH, trophic status, and hardness, reflecting a pre-existing gradient in water chemistry. Biomagnification of MeHg (as determined by regression of MeHg concentration on baseline-adjusted $\delta^{15}N$) was negatively correlated with lake water chlorophyll *a* concentration. A subsequent logging experiment interrupted by fire provided an opportunity to compare pre- and post-fire MeHg concentrations in aquatic biota. Three months after fire, biota MeHg concentrations had decreased by 32%–50%, and lake water ammonium concentrations had increased 11-fold relative to the previous year. In this nutrient-rich setting, fire may lower MeHg concentrations in aquatic biota over the short-term by inducing an increase in lake productivity that dilutes MeHg at the base of the food web.

Résumé : Nous avons comparé les concentrations de méthyl-mercure (MeHg) dans les macroinvertébrés et les poissons dans cinq lacs à bassins versants incendiés et dans cinq lacs témoins dans la plaine boréale de l'Ouest canadien afin de déterminer l'influence des feux de forêt sur la bioconcentration de MeHg. Deux années après le feu, les concentrations de MeHg chez cinq de six taxons aquatiques sont semblables dans les lacs affectés par le feu et les lacs témoins. Dans une série plus grande de 12 lacs, il y a une corrélation négative entre les concentrations de MeHg dans les organismes vivants et le pH de l'eau du lac, le statut trophique et la dureté, ce qui reflète l'existence d'un gradient préexistant de chimie de l'eau. La bioconcentration de MeHg (déterminée par la régression des concentrations de MeHg sur un $\delta^{15}N$ de base ajusté) est en corrélation négative avec la concentration de chlorophylle *a* dans l'eau du lac. Une expérience subséquente de coupe de forêt interrompue par un feu a fourni l'occasion de comparer les concentrations de MeHg avant et après le feu chez les organismes aquatiques. Trois mois après le feu, les concentrations de MeHg des organismes avaient décru de 32 à 50 % et les concentrations d'ammonium dans l'eau de lac avaient augmenté par un facteur de 11, par rapport à l'année précédente. Dans ces conditions d'abondance de nutriments, le feu peut réduire à court terme les concentrations de MeHg dans les organismes en provoquant un accroissement de la productivité lacustre qui dilue MeHg à la base du réseau alimentaire.

[Traduit par la Rédaction]

Introduction

Methyl mercury (MeHg) is a pollutant of concern in aquatic ecosystems due to its toxicity and high rate of bioaccumulation (World Health Organization 1990). Recent studies from Canada's Boreal Shield reported higher MeHg concentrations in zooplankton and fish from lakes with logged watersheds than in undisturbed watersheds (Garcia and Carignan 1999, 2000). The same studies reported no influence of forest fire on MeHg concentrations in aquatic biota. These findings have important implications for the natural disturbance paradigm of forest management, which proposes that timber harvesting can emulate the ecological impacts of natural disturbances such as forest fire (see Seymour et al. 2002).

Watershed disturbance may influence MeHg dynamics in lakes by increasing soil erosion and export of terrestrial mercury (Hg) to surface waters, or by altering lake water chemistry. Soil-bound Hg is mobilized by the leaching of dissolved organic carbon (DOC; Mierle and Ingram 1991), a

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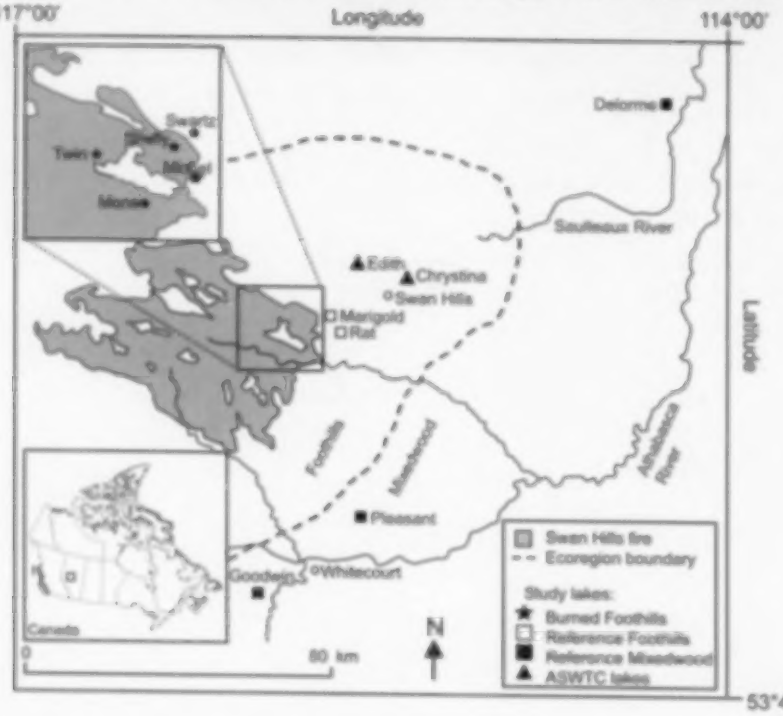
Fig. 1. Study area in the province of Alberta, Canada (inset). ASWTC, Alberta Special Waste Treatment Centre.



process that can be promoted by forest fire (McEachern et al. 2000) and clear-cutting (Carignan et al. 2000). Increased inputs of allochthonous DOC can reduce lake water pH and light penetration, which in turn may increase net methylation rates (Miskimmin et al. 1992; Sellers et al. 1996). Alternatively, if disturbance-related inputs of nutrients increase algal biomass (e.g., Carignan et al. 2000), MeHg concentrations in aquatic biota may decrease as a result of biodilution (Larsson et al. 1992; Pickhardt et al. 2002). Few studies have addressed the influence of watershed disturbance on MeHg bioaccumulation, and it remains unclear whether the findings of Garcia and Carignan (1999, 2000) apply to other regions, such as the western Boreal Plain.

The Boreal Plain represents 25% of the Canadian boreal forest and, unlike the Boreal Shield, is characterized by deep glacial tills, a moderately dry climate, and shallow productive lakes (Mitchell and Prepas 1990). To date there have been no comprehensive studies on MeHg dynamics in Boreal Plain lakes, despite numerous fish consumption advisories (Alberta Environment 2000). Watershed disturbance is an increasing concern in the region due to the expansion of timber-harvest activities and the construction of roads, seismic lines, and other infrastructure needed for oil and natural gas exploration.

Forest fires are an important source of watershed disturbance on the Boreal Plain (Johnson et al. 1998), and can provide a natural context for the impacts of forest canopy removal on surface-water chemistry (e.g., McEachern et al. 2000; Allen et al. 2003). Dry conditions in recent years, combined with forest management practices of fire suppression in the second half of the 20th century, have contributed to an increasing number of large-scale (>100 000 ha) forest fires throughout the region. In May 1998, a wildfire burned 150 000 ha of Boreal Plain forest in the Swan Hills Upland, 200 km northwest of Edmonton, Alberta (Fig. 1). The Swan Hills is a region of toxicological concern due to emissions from the Alberta Special Waste Treatment Centre (ASWTC), a hazardous waste incineration facility that has operated since 1987. High MeHg and dioxin concentrations in wildlife near the ASWTC have led to a wildlife consumption ban within 30 km of the facility (Alberta Environment 2000; Blais et al. 2003), although to date, no published studies have linked high MeHg concentrations in fish from nearby lakes to emissions from the ASWTC. The Swan Hills fire provided an opportunity to determine the short-term impacts of forest fire on MeHg concentrations in aquatic biota, in a region of the Boreal Plain where MeHg bioaccumulation is a human health issue.

In this study, we compare MeHg concentrations in macroinvertebrates and fish from burned lakes with those from nearby reference lakes. In a separate comparison, MeHg concentrations in biota from a single lake are compared before and after a forest fire interrupted a prescribed timber harvest experiment. As a further objective, we examine the influence of lake water chemistry, watershed characteristics, and trophic ecology on the bioaccumulation and biomagnification of MeHg in littoral food webs.

Materials and methods

Study area

The study area spanned two ecoregions in the Swan Hills region of west-central Alberta: the Boreal Foothills (upper

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elevations) and the Boreal Mixedwood (lower elevations; Strong and Leggat 1992; Fig. 1). Upper elevations are dominated by lodgepole pine (*Pinus contorta*), white and black spruce (*Picea glauca*, *Picea mariana*), and balsam fir (*Abies balsamea*). Mixedwood stands at lower elevations consist of trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), and white spruce. Bogs and fens cover ~5% of the landscape. Lakes are relatively scarce, small (<40 ha), and shallow (mean depth <3 m), and range from oligotrophic at high elevations to eutrophic at lower elevations (Allen et al. 2003). Mean summer rainfall (May–August, 1945–1990) ranges from 340 mm at a 540-m elevation to 458 mm at a 1400-m elevation (Alberta Sustainable Resource Development, Main Floor 9920 108 Street, Edmonton, AB T5K 2M4, Canada, unpublished data). Long-term (1972–1997) annual evaporation is 90% of annual precipitation at the nearest monitoring site (Whitecourt, 850 m; Environment Canada 2002).

The study design comprised five burned and five reference lakes, as well as two lakes near the ASWTC (Table 1). Both ASWTC lakes have fish consumption advisories due to high Hg concentrations. Lake selection criteria were as follows: (*i*) headwater lakes, (*ii*) upland-dominated drainage basins (<25% wetlands), (*iii*) 10–40 ha in size, (*iv*) maximum depth > 2 m, and (*v*) drainage ratio (DR; drainage basin area (DBA)/lake area) > 2. All burned lakes and the ASWTC lakes are situated in the Boreal Foothills portion of the study area. Only two reference lakes matching the selection criteria were found in the Foothills portion of the study area. Three additional reference lakes, including one lake selected for experimental timber harvest (Delorme), were selected from lower elevations in the Boreal Mixedwood ecoregion (Fig. 1). Similar to most forested portions of the Boreal Plain, all study catchments had linear disturbances (e.g., cutlines and roads) affecting <2% of the DBA, and the Moms Lake (burned) and Edith Lake (ASWTC) drainage basins each had an oil well installation. Edith Lake and Chrystina Lake are 10 km west and 1.5 km north of the ASWTC, respectively, whereas burned and reference lakes are situated over 30 km from the ASWTC. ASWTC lakes were not included in the burned versus reference comparisons.

As reported by Allen et al. (2003), reference Mixedwood lakes had higher pH and total phosphorus (TP), chlorophyll *a* (chl *a*), DOC, and dominant cation concentrations than that of reference Foothills lakes. Consequently, in a comparison of burned versus reference water chemistry, the high trophic status of the reference Mixedwood lakes may have concealed a post-fire increase in nutrient concentrations in the relatively dilute Foothills lakes. When the comparison was made by ecoregion (i.e., burned Foothills versus reference Foothills) there were indications of a fire effect: 2 years after fire, burned Foothills lakes had 1.4-fold higher DOC concentrations than reference Foothills lakes, and lake water colour was positively correlated with the ratio of DBA burned to lake volume (see Allen et al. 2003).

Given pre-existing differences in water chemistry between Mixedwood and Foothills lakes, the inclusion of Mixedwood lakes as reference catchments in our study poses a potential bias towards finding a post-fire increase in biota MeHg concentrations (i.e., based on pre-existing conditions, MeHg concentrations in Mixedwood lakes should be lower than MeHg in Foothills lakes regardless of disturbance). However, the inclusion of Mixedwood lakes was justified for the following reasons: (*i*) industrial partners identified Mixedwood catchments as the most feasible for prescribed timber harvest of a lake watershed, and selection of an experimental watershed in the Mixedwood ecoregion necessitated the inclusion of similar systems as reference lakes; (*ii*) anthropogenic disturbance is widespread in the study area, and together with the low density of lakes, made it difficult to select additional catchments in the Foothills; and (*iii*) Mixedwood catchments are representative of the dominant lake type on the Boreal Plain.

In a prescribed clearcutting experiment, the drainage basin of Delorme Lake (reference Mixedwood) was 50% clear-cut in the winter of 2000–2001 after pre-disturbance sampling in 2000. However, the experiment was disrupted in May 2001 by the large-scale (116 000 ha) Chisholm fire. Although the clear-cut experiment was abandoned, the fire permitted us to expand our examination of the influence of forest fire on MeHg bioaccumulation to include two timeframes: (1) a burned versus reference comparison at Year 2 post-fire (1998 Swan Hills fire; hereafter referred to as the post-fire survey) and (2) a pre- versus post-disturbance comparison at 3 months post-fire in Delorme Lake (2001 Chisholm fire, hereafter referred to as the pre- versus post-fire survey). The Goodwin Lake drainage basin, which was undisturbed between 2000 and 2001 and had similar deciduous cover to the Delorme watershed, served as a reference for post-disturbance conditions at Delorme Lake.

Collection of biota

Macroinvertebrates and fish were collected from all lakes in September 2000 and from Delorme and Goodwin lakes in late August 2001. Macroinvertebrates were collected by sweeping macrophytes and surficial sediments with a 1000-µm pond net. Brook stickleback (*Culaea inconstans*) and pearl dace (*Margariscus margarita*) were collected with Gee minnow traps set 1–3 m from shore at depths of 0.5–1 m. Common macroinvertebrate taxa representing a range of feeding strategies were collected from 10 littoral sites randomly selected along the entire shoreline: *Agrypnia* spp., and *Phryganea* spp. (Trichoptera: Phryganeidae), *Gammarus lacustris* (Crustacea: Amphipoda), *Aeshna* spp. (Odonata: Aeshnidae), *Cordulia shurtleffi* (Odonata: Corduliidae), and *Notonecta* spp. (Hemiptera: Notonectidae). Taxa were distributed unevenly among lakes (Table 2). The two Trichoptera genera appeared to be mutually exclusive in the lakes and were treated as one family. The snail *Physa* spp. (Gastropoda: Physidae) was collected to adjust for baseline variation in stable nitrogen isotope ratios ($\delta^{15}N$; Post 2002), but was not analyzed for MeHg. Pearl dace were only found in Delorme Lake (Table 2), and were only included in the pre- versus post-fire survey.

Macroinvertebrates and fish were placed in plastic buckets filled with lake water and live sorted within 2 h of collection. Macroinvertebrates were then placed in separate pans filled with deionized distilled water (DDW) for 12–15 h to allow for defecation of gut contents, then blotted on Whatman filter paper. Fish and macroinvertebrates were sealed in sterile Whirl-Pak® bags and frozen at –20 °C on dry ice.

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Table 1. Drainage basin and lake characteristics.

Lake	Ecoregion	LA (ha)	CA (ha)	CA/LV (m^{-1})	Z_m (m)	ELEV (m)	CON (%)	WET (%)	BURN (%)	pH	DOC ($mg \cdot L^{-1}$)	Colour ($mg\ Pt \cdot L^{-1}$)	TP ($\mu g \cdot L^{-1}$)	CHL *a* ($\mu g \cdot L^{-1}$)	COND ($\mu S \cdot cm^{-1}$)
Burned															
Mons	Foothills	19	55	2.6	1	990	90	7	90	9	12	36	28	4.4	82
Twin	Foothills	13	143	7.8	1	1040	88	10	70	7	17	139	23	5.6	28
Shelly	Foothills	23	128	2.8	2	1100	57	11	80	8	9	29	24	4.8	88
Michel	Foothills	13	133	7.2	1	990	63	10	50	7	16	71	19	4.4	61
Swartz	Foothills	28	328	5.4	2	1090	86	7	20	8	12	40	31	7.3	94
Mean Burned		19	157	5.2	2	1042	77	9	62	7	13	63	25	5.3	71
Reference															
Rat	Foothills	10	128	13	1	1095	80	9		8	11	34	24	3.7	84
Marigold	Foothills	34	96	1.8	2	1115	87	13		8	6	12	16	2	57
Pleasant	Mixwood	18	254	6.9	2	775	4	2		9	14	16	34	13.8	138
Goodwin	Mixwood	18	253	6.5	2	860	32	3		8	12	41	32	14.1	202
Delorme	Mixwood	34	285	4.8	2	610	40	10		9	32	72	38	19.1	246
Mean Reference		25	209	6.6	1.6	891	49	7		7	15	35	29	10.5	145
ASWTC															
Christina	Foothills	39	261	1.7	4	1015	95	4		7	8	17	20	5.2	32
Edith	Foothills	22	77	2.8	1	995	80	17		7	11	46	16	3.7	64
Mean ASWTC		30	169	2.2	3	1005	88	11		7	9.3	32	18	4.4	48
Overall		22	178	5.2	2	973	67	9	—	8	13	46	25	7.3	98

Note: LA, lake area; CA, catchment area; LV, lake volume; Z_m, mean depth; ELEV, elevation; CON, conifer cover; WET, wetland cover; BURN, percent burned; DOC, dissolved organic carbon; TP, total phosphorus; CHL *a*, chlorophyll *a*; COND, conductivity; ASWTC, Alberta Special Waste Treatment Centre.

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Table 2. Number of study lakes in which macroinvertebrate and fish taxa occurred.

Taxon	Feeding strategy	Occurrence in study lakes				
		Reference		Burned foothills	ASWTC	Total
		Mixedwood	Foothills			
Macroinvertebrates						
Physa spp.	Grazer	3	2	5	2	12
Phryganeidae	Detritivore/shredder	3	2	4	1	10
Gammarus lacustris	Detritivore/collector	3	1	3	-	7
Cordulia shurtleffi	Predator/engulfer	2	1	3	2	8
Aeshna spp.	Predator/engulfer	2	1	4	2	9
Notonecta spp.	Predator/piercer	3	2	5	2	12
Fish						
Culaea inconstans	Planktivore/benthivore	3	1	2	2	8
Margariscus margarita	Planktivore/benthivore	1	—	—	—	1

Note: ASWTC, Alberta Special Waste Treatment Centre; dash (—) indicates not present.

Chemical analyses

Collection and chemical analyses of lake water and drainage basin morphometry for the Swan Hills lakes are detailed in Allen et al. (2003). With the exception of Delorme (sampled August and October 2000, biweekly June–October 2001) and Edith lakes (sampled August 2000), water samples were collected once in each of July and August 2000.

Macroinvertebrates were wet weighed and body lengths measured prior to freeze-drying for 70 h at –60 °C and 100 millitorr (1 millitorr = 0.1332 Pa). With the exception of odonates, which were analyzed as individuals, 5–15 individuals of each macroinvertebrate taxon were pooled to meet the required mass for MeHg and stable isotope analyses. Dried samples were homogenized in Whirl-Pak® bags and between 50 and 100 mg of each homogenate was transferred to glass vials with Teflon-lined closures. Where possible, at least 10 aggregate or individual samples were analyzed for MeHg and $\delta^{15}N$ per taxon per lake (mean = 9 ± 0.4 samples; range = 2–12). Snails were analyzed for $\delta^{15}N$ as individuals (mean of 6 ± 0.3 individuals per lake; range = 4–7).

Fish were measured for total length, weighed, sexed, eviscerated, and grouped by 1- to 5-cm size intervals into aggregate, whole-body samples of 3–5 males of each species (mean of 9 ± 0.6 aggregate samples per lake; range = 6–12). Mean total lengths of aggregate brook stickleback samples ranged within lakes from 48 ± 0.8 mm to 62 ± 3 mm (n = 8). This size range was assumed to include 1- and 2-year-old fish based on life history data of a brook stickleback population in a small, shallow prairie lake (Moodie 1986). Mean total lengths of pearl dace aggregate samples from Delorme Lake ranged in size from 56 ± 4 to 72 ± 5 mm (mean of pre- and post-fire years). Fish were freeze-dried prior to homogenization in a stainless steel blender cup. Between 200 and 400 mg of homogenate from each aggregate sample was transferred to glass vials and shipped to Flett Research Ltd. (Winnipeg, Manitoba).

To prevent contamination, all glassware and polyethylene containers were soaked in 10% HNO_3 (trace metals grade) and thoroughly rinsed with DDW. MeHg in tissue was detected with cold vapour atomic fluorescence spectrophotometry at Flett Research Ltd. (Winnipeg, Manitoba). Detection of MeHg followed overnight digestion in KOH methanol, ethylation with tetraethyl borate, collection on Tenax traps, and separation on a gas chromatograph (Horvat et al. 1993). The average coefficient of variation (CV) for duplicate analyses, performed on 10% of the samples (n = 74), was 8.1 ± 0.7%. Repeated analyses (two digestions prepared) were also performed on 10% of the samples (n = 80) with an average CV of 7.1 ± 0.6%. Standard materials (DORM-2) were run approximately every eight samples to determine the recovery of MeHg (mean recovery = 86 ± 0.5%; n = 135). MeHg concentrations were based on DORM-2 recoveries and reported as $ng \cdot g^{-1}$ dry weight.

Time-integrated estimates of trophic position were estimated with stable isotope ratios of nitrogen (^{15}N:^{14}N; Peterson and Fry 1987). Stable isotope ratios were calculated as the parts per million (‰) deviation from standard material:

$$(1) \qquad \delta^{15}N = ([R_{sample}/R_{standard}] - 1) \times 1000$$

where $R = {}^{15}N/{}^{14}N$. Atmospheric nitrogen was used as the reference material for $\delta^{15}N$, and $R_{standard}$ values were set at 0‰. For all tissues, 1.2–1.7 mg of material was packed into 8 mm × 5 mm tin capsules. Macroinvertebrate tissue was subsampled from homogenates prepared for MeHg analysis. Snail foot muscle tissue was dissected from the shell, freeze-dried, and homogenized with a glass mortar and pestle. Fish dorsal muscle was dissected from each individual in the aggregate samples prepared for MeHg analysis. The muscle samples were freeze-dried for 50 h at –60 °C, homogenized, and packed in tin capsules. Stable isotope ratios were measured with a Micromass Optima continuous-flow mass spectrometer coupled to a Carlo Erba Na1500 elemental analyzer and Autosampler. Standards were analyzed every 10 samples; the average CV for duplicate samples (n = 14) was 9.6 ± 2.0%. To adjust for baseline variation in $\delta^{15}N$, gastropod $\delta^{15}N$ values were subtracted from consumer $\delta^{15}N$ (Post 2002).

Statistical analyses

Post-fire survey

MeHg concentrations in biota were compared between burned and reference lakes with the Mann–Whitney U test. The conservative Mann–Whitney test was used because of the low number of replicates for some groups (n ranged from 2 to 5). Mean trophic positions of each taxon were also

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compared between burned and reference lakes with the Mann–Whitney *U* test. The burned versus reference comparison was biased somewhat by pre-existing differences in water chemistry between Mixedwood and Foothills lakes; however, low sample sizes prevented a proper analysis of covariance and subsequent adjustment of MeHg means to account for these differences. Instead, we interpreted our results in the context of Pearson's correlation coefficients calculated for the entire lake set between lake mean MeHg concentrations in biota and 28 abiotic and biotic variables (pH, conductivity, alkalinity, TP, total dissolved phosphorus (TDP), chl *a*, DOC, colour, dissolved inorganic nitrogen (DIN), Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, conifer cover, wetland cover, lake area (LA), catchment area (CA), lake volume (LV), CA/LV, elevation, mean and maximum lake depth, individual dry weight and total length, and trophic position). Within-lake relationships between fish MeHg concentration and total length were also tested with the Pearson's correlation coefficient.

Biomagnification was examined by linear regression of $\log_{10}$MeHg concentrations on $\delta^{15}N$ for each lake. Biomagnification rate (i.e., the slope of the $\log_{10}$MeHg versus $\delta^{15}N$ relationship) was compared among lakes with analysis of covariance (ANCOVA). Covariation of biomagnification rates and environmental variables were tested with Pearson's correlation coefficients among lakes in which biota spanned three trophic levels (i.e., Trichoptera to brook stickleback).

Pre- versus post-fire comparison

Pre- and post-fire MeHg concentrations, trophic positions, and body sizes of Delorme Lake biota were compared using Student's *t* test on a taxon-by-taxon basis. Given the short life spans of the sampled organisms, we assumed that their MeHg burdens reflected lake conditions for the year in which they were collected, and that post-fire MeHg concentrations were effectively independent of pre-fire MeHg concentrations. Water chemistry variables were compared using the Mann–Whitney *U* test because of low sample sizes (pre-fire, $n = 2$ or 3; post-fire, $n = 5$).

Variables were tested for normality with the Shapiro–Wilkes test, and non-normal variables were $\log_{10}$ transformed. Proportions were arcsine transformed. Variables that were not normalized by transformation were analyzed with nonparametric methods. Statistical analyses were conducted with SPSS (v. 8.0; SPSS Inc., Chicago, Illinois). With the exception of Pearson's correlation coefficients, results were considered significant at $P \leq 0.05$. To account for repeated tests with the lake chemistry – watershed characteristics dataset (six for each taxon and one for biomagnification rate), the α value was lowered to 0.007 (0.05/7). Variances cited are 1 standard error of the mean.

Results

Post-fire survey

With the exception of the odonate *Cordulia shurtleffi*, MeHg concentrations in biota were similar between burned and reference lakes (Fig. 2). MeHg concentration in *Cordulia shurtleffi* was 1.5-fold higher in burned lakes (55 ± 4 ng·g^{-1}, $n = 3$) than in reference lakes (38 ± 6 ng·g^{-1}, $n = 3$; Mann–Whitney *U* test, $P = 0.05$). Mean MeHg concentrations in biota from burned and reference lakes, respectively, were as follows: Phryganeidae, 16 ± 2 ng·g^{-1} ($n = 4$) and 14 ± 1 ng·g^{-1} ($n = 5$; $P = 0.5$); *Gammarus lacustris*, 32 ± 2 ng·g^{-1} ($n = 3$) and 27 ± 4 ng·g^{-1} ($n = 4$; $P = 0.3$); *Aeshna* spp., 84 ± 5 ng·g^{-1} ($n = 2$) and 83 ± 36 ng·g^{-1} ($n = 4$; $P = 0.5$); *Notonecta* spp., 175 ± 12 ng·g^{-1} ($n = 5$) and 172 ± 18 ng·g^{-1} ($n = 5$; $P = 0.9$); and brook stickleback 153 ± 14 ng·g^{-1} ($n = 2$) and 110 ± 26 ng·g^{-1} ($n = 4$; $P = 0.4$). Baseline-adjusted $\delta^{15}N$ values were similar between burned and reference lakes for all taxa, indicating that, on average, organisms from each taxon occupied similar trophic positions in the two sets of lakes (Mann–Whitney *U* test, $0.06 > P < 0.9$; Table 3). Brook stickleback $\delta^{15}N_{adj}$ were slightly elevated in reference lakes ($P = 0.06$), but the difference (0.7‰) was considered negligible given the 3.4‰ increase per trophic level.

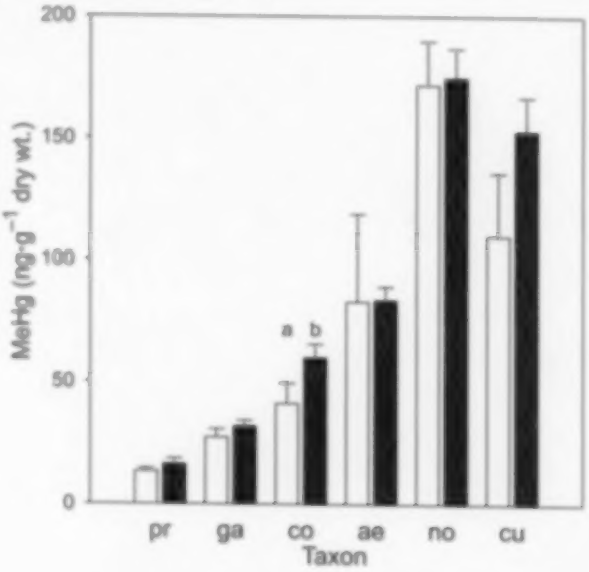

Fig. 2. Comparison of mean methyl mercury (MeHg) concentrations in biota from burned (solid bars) and reference lakes (open bars). Letters indicate differences between treatments (Mann–Whitney *U* test, $P < 0.05$). Taxon codes: pr, Phryganeidae; ga, *Gammarus lacustris*; co, *Cordulia shurtleffi*; ae, *Aeshna* spp.; no, *Notonecta* spp.; cu, *Culaea inconstans*. Error bars represent +1 standard error of the mean.

MeHg concentrations in biota varied widely among individual lakes (including the ASWTC lakes; Fig. 3), reflecting water chemistry, watershed characteristics, and trophic factors (Table 4). With the exception of *Notonecta*, MeHg concentrations in biota from reference Mixedwood lakes were relatively low, whereas concentrations in biota from the ASWTC lakes, in particular Edith Lake, were relatively high.

Phryganeidae MeHg concentrations ranged among lakes from 10 ± 1 ng·g^{-1} to 21 ± 2 ng·g^{-1} (Fig. 3), and were most strongly correlated with trophic position ($\delta^{15}N_{adj}$; $r = 0.88$, $P < 0.001$, $n = 10$) (Table 3). Baseline-adjusted $\delta^{15}N$ values for Phryganeidae ranged among lakes from –4.3‰ to 1.1‰, spanning an entire trophic level. This may reflect a shift in the diet of Phryganeidae among lakes from shredders to

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Fig. 3. Mean methyl mercury (MeHg) concentrations in macroinvertebrates and fish from burned (solid bars), reference Foothills (open bars), reference Mixedwood (shaded bars), and lakes near the Alberta Special Waste Treatment Centre (hatched bars). Lake abbreviations: mo, Mons; tw, Twin; sh, Shelly; mi, Michel; sw, Swartz; ra, Rat; ma, Marigold; pl, Pleasant; go, Goodwin; de, Delorme; ch, Chrystina; ed, Edith. Error bars represent +1 standard error of the mean.

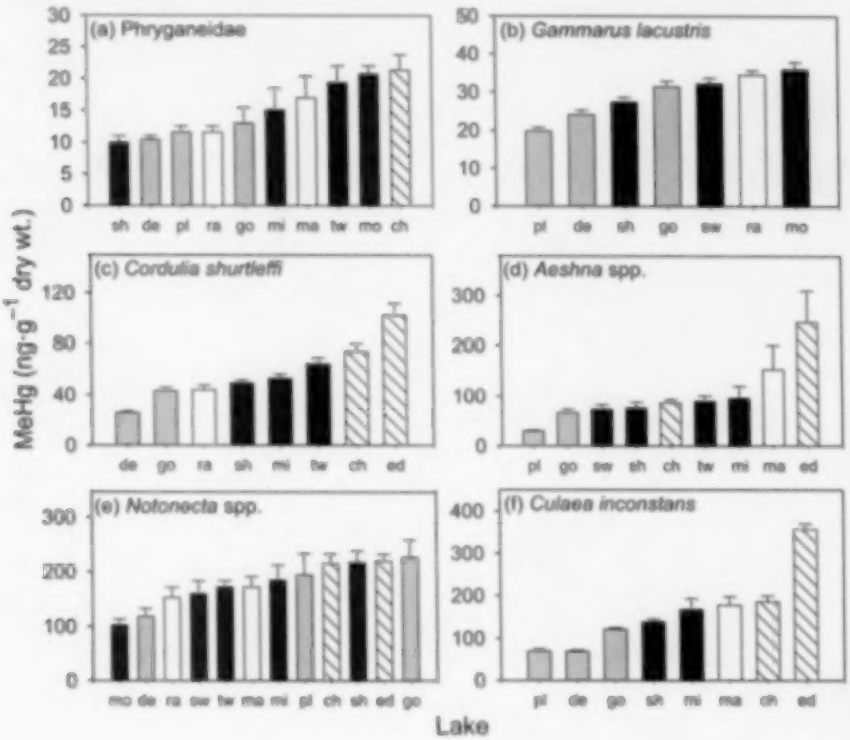

Table 3. Mean baseline-adjusted $\delta^{15}N$ values for taxa in burned, reference, and Alberta Special Waste Treatment Centre (ASWTC) lakes.

Taxon	Baseline adjusted $\delta^{15}N$ (‰)			
	Burned	Reference	ASWTC	*P*
Macroinvertebrates				
Physa spp.	2.4±0.2	2.4±0.4	3.7±0.6	0.9
Phryganeidae	–1.9±0.7	–2.8±0.6	1.1	0.33
Gammarus lacustris	0.0±0.5	–0.4±0.2	—	0.29
Cordulia shurtleffi	0.4±0.1	0.4±0.1	1.0±0.1	0.83
Aeshna spp.	1.5±0.2	1.5±0.1	2.4±0.3	0.72
Notonecta spp.	1.6±0.1	1.5±0.1	0.7±0.6	0.92
Fish				
Culaea inconstans	4.9±0.1	5.6±0.3	6.3±0.0	0.06

Note: *Physa* spp. $\delta^{15}N$ values are not baseline adjusted; *P* value from Mann–Whitney *U* test (burned versus reference); errors reported are 1 standard error of the mean; baseline-adjusted $\delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{Physa}$.

predators of aquatic insects and crustaceans (Wiggins 1998). MeHg concentrations in Phryganeidae also increased with percentage of conifer cover (arcsin CON; $r = 0.74$, $P = 0.007$), and decreased with $\log_{10}$ conductivity ($r = -0.68$, $P = 0.02$) and $\log_{10} Mg^{2+}$ concentration ($r = -0.64$, $P = 0.02$).

MeHg concentrations in *G. lacustris* ranged among lakes from 20 ± 1 ng·g^{-1} to 36 ± 2 ng·g^{-1}. Similar to Phryganeidae, *G. lacustris* MeHg concentrations increased with percentage of conifer cover (arcsinCON, $r = 0.87$, $P = 0.006$, $n = 7$). Weaker, negative correlations were detected between *G. lacustris* MeHg concentration and mean body length ($r = -0.84$, $P = 0.01$) and $\log_{10}$chl *a* concentration ($r = -0.68$, $P = 0.05$), reflecting an increase in body size and decrease in MeHg concentrations with increasing lake trophic status.

MeHg concentrations in odonates and brook stickleback were strongly correlated with lake water chemistry, in particular pH, and indices of lake trophic status. *Cordulia shurtleffi* MeHg concentrations ranged from 26 ± 1 ng·g^{-1} to 102 ± 10 ng·g^{-1}, and were correlated with pH ($r = -0.94$, $P < 0.001$, $n = 10$; Fig. 4), TP ($r = -0.88$, $P = 0.002$) and water hardness indices (e.g., $\log_{10} Ca^{2+}$, Mg^{2+}; see Table 2). *Aeshna* MeHg concentrations ranged from 30 ± 2 ng·g^{-1} to 247 ± 64 ng·g^{-1}. Similar to *Cordulia*, MeHg concentrations in *Aeshna* were negatively correlated with pH ($r = -0.81$, $P = 0.004$, $n = 9$), as well as with lake trophic status and water hardness indices (Table 3). The strongest correlate of MeHg in *Aeshna* was wetland cover ($r = 0.87$, $P = 0.001$), which ranged from 2% to 17% of the catchments in which *Aeshna* were collected.

Notonecta had the highest MeHg concentrations among all biota sampled, ranging among lakes from 118 ± 14 ng·g^{-1} to 219 ± 13 ng·g^{-1}. There were no significant correlations between *Notonecta* MeHg concentrations and lake chemistry –

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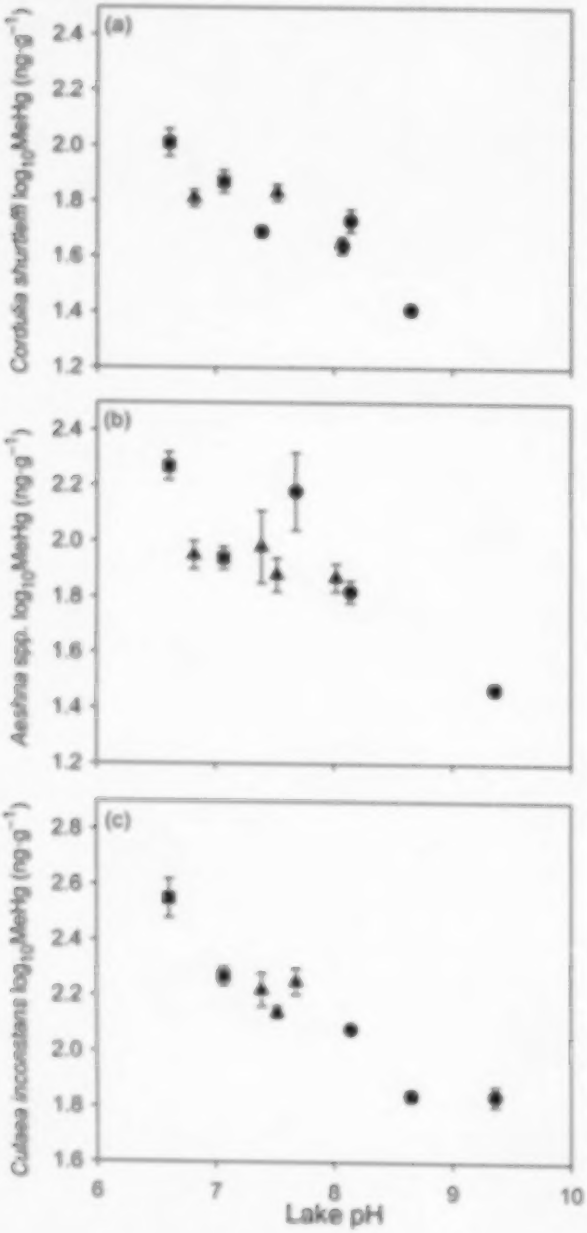
Fig. 4. Scatterplots of lake pH and $\log_{10}$ methyl mercury (MeHg) concentration in (*a*) *Cordulia shurtleffi*, (*b*) *Aeshna* spp., and (*c*) *Culaea inconstans* from burned (▲), reference (●), and Alberta Special Waste Treatment Centre (■) lakes. Error bars represent ±1 standard error of the mean.



watershed variables. A weak negative correlation with $\log_{10}$TDP ($r = -0.64$, $P = 0.01$, $n = 12$) was the only indication that MeHg bioaccumulation in *Notonecta* followed the patterns exhibited by other taxa.

MeHg concentrations in brook stickleback ranged among lakes from 69 ± 3 ng·g^{-1} to 357 ± 13 ng·g^{-1}. MeHg concentrations in brook stickleback were inversely correlated with pH ($r = -0.94$, $P < 0.001$, $n = 8$) and TP ($r = -0.90$, $P = 0.001$). Weaker inverse correlations were also detected for deciduous cover and water hardness indices (Table 3). With the exception of Marigold Lake (reference Foothills), there were no correlations between MeHg concentrations in brook stickleback and total length within lakes. In Marigold Lake, MeHg concentrations in stickleback were negatively correlated with body size ($r = -0.73$, $P = 0.04$, $n = 8$), contrary to positive correlations previously reported for other fish species (e.g., Grieb et al. 1990). Therefore differences in total length of brook stickleback among lakes (lake means = 45–61 mm, analysis of variance (ANOVA); $F = 4.8$; degrees of freedom (df) = 7, 85; $P < 0.01$) likely did not contribute to among-lake variation in stickleback MeHg concentrations.

MeHg biomagnification

Stable N isotope analysis identified a trophic gradient that spanned up to three trophic levels within lakes (assuming 3.4‰ δ^{15}N per trophic level), extending from Phryganeidae (lake mean $\delta^{15}N_{adj} = -2.0 \pm 0.5$‰) through *G. lacustris* (–0.2 ± 0.2‰), *Cordulia shurtleffi* (0.6 ± 0.1‰), *Aeshna* (1.4 ± 0.1‰), *Notonecta* (1.7 ± 0.2‰), to stickleback (5.6 ± 0.2‰). When *Notonecta* were excluded, linear relationships were detected between $\log_{10}$MeHg and δ^{15}N of biota in all 12 lakes ($0.57 > r^2 < 0.91$, $P < 0.001$; Fig. 5). *Notonecta* were excluded because their MeHg concentrations were clearly higher than expected based on their δ^{15}N values; this was particularly evident in Shelly Lake, Pleasant Lake, Goodwin Lake, Delorme Lake, and Chrystina Lake (Figs. 5*c*, 5*g*, 5*h*, 5*i*, and 5*k*). Biomagnification rates (i.e., slopes of the $\log_{10}$MeHg versus δ^{15}N relationship) varied among lakes from 0.07 to 0.29 (ANCOVA, $F = 12$; df = 11, 353; $P < 0.001$). Among lakes in which biota spanned three trophic levels, biomagnification rate was inversely correlated with $\log_{10}$chl *a* ($r = -0.90$, $P = 0.006$, $n = 7$; Fig. 6). Weaker correlations were detected between biomagnification rate and elevation ($r = 0.86$, $P = 0.01$) and $\log_{10}$TP ($r = -0.85$, $P = 0.02$). Thus MeHg biomagnification appeared to be inversely related to lake trophic status.

Pre- versus post-fire comparison

MeHg concentrations in biota from Delorme Lake declined considerably after winter logging and a spring forest fire (Fig. 7). Relative to pre-fire values, MeHg concentrations in biota decreased by 50% in Phryganeidae (Student's *t* test, $P < 0.001$, df = 22), 43% in *G. lacustris* ($P < 0.001$, df = 22), 32% in *Cordulia shurtleffi* ($P = 0.007$, df = 21), 36% in pearl dace ($P = 0.04$, df = 22), and 42% in brook stickleback ($P < 0.01$, df = 21). Over the same time period, MeHg concentrations in the three common taxa (*G. lacustris*, *Cordulia shurtleffi*, and brook stickleback) from Goodwin Lake (reference Mixedwood) were unchanged ($P > 0.3$, $17 > df < 20$).

Mean body sizes and δ^{15}N of several taxa from Delorme Lake differed slightly between pre- and post-fire samples. δ^{15}N signatures in Phryganeidae and *G. lacustris* increased by 0.9‰ and 0.5‰, respectively (Student's *t* test, $P < 0.001$), possibly reflecting increased input of dissolved inorganic nitrogen (DIN; Lake et al. 2001). Mean body weights of Phryganeidae and *G. lacustris* were 6% and 13% higher, respectively, in 2001 than in 2000 ($P < 0.05$ and < 0.001).

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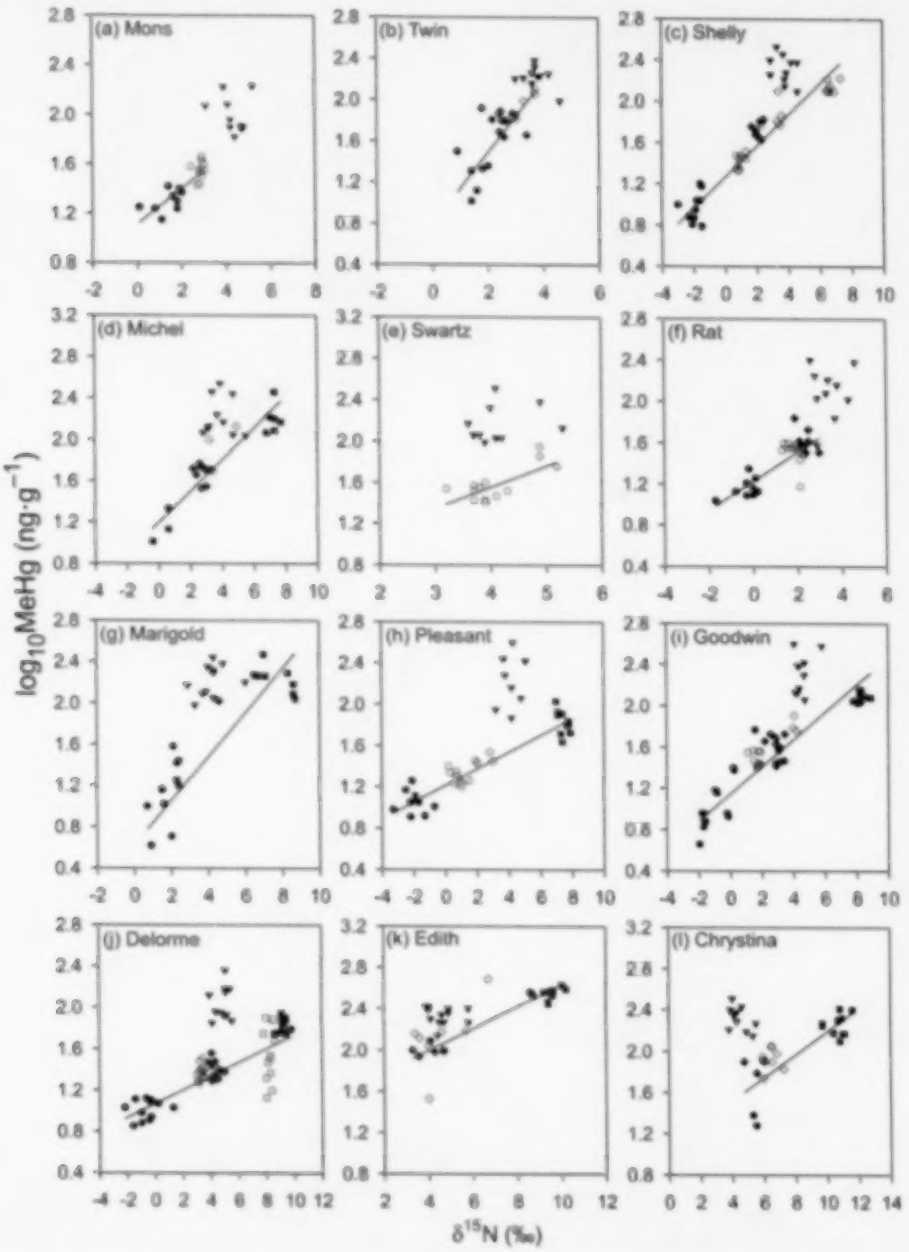
Fig. 5. Linear regressions of $\log_{10}$ methyl mercury (MeHg) concentration on baseline-adjusted $\delta^{15}N$ ($\delta^{15}N_{adj}$) in Phryganeidae (○), *Gammarus lacustris* (●), *Cordulia shurtleffi* (◆), *Aeshna* spp. (◇), pearl dace (*Margariscus margarita*; □), and brook stickleback (*Culaea inconstans*; ■). Regression statistics: *Notonecta* spp. (▼) were not included in the regressions ($26 > F < 415$, $0.57 > r^2 < 0.91$, $P < 0.003$, $19 > df < 58$).



Also, body weight to length ratios increased by 14% in Phryganeidae, and 40% in *G. lacustris* ($P = 0.02$ and <0.001). There were no differences in body size or $\delta^{15}N$ in *Cordulia shurtleffi*, pearl dace, and brook stickleback between pre- and post-fire years.

The decrease in MeHg concentration in Delorme Lake biota was accompanied by changes in lake water chemistry, most notably an increase in ammonium (NH_4^+) concentrations (Table 5). Post-fire concentrations of NH_4^+ in Delorme Lake were elevated throughout the summer, rising to between 80 ng·g^{-1} and 160 µg·g^{-1} during major rain events (>20 mm·day^{-1}). Between August and October, post-fire concentrations of NH_4^+ were 11-fold higher than pre-fire concentrations ($n = 5$ and 2, respectively; Mann–Whitney *U*

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Fig. 6. Scatterplot of methyl mercury (MeHg) biomagnification rate (slope of the $\log_{10}$MeHg (ng·g^{-1}) versus $\delta^{15}N_{adj}$ (‰) regression) and $\log_{10}$ chlorophyll *a* (chl *a*) concentration in burned (▲), reference Foothills (●), reference Mixedwood (○), and Alberta Special Waste Treatment Centre (■) lakes in which biota spanned three trophic levels. Pearson's correlation coefficient, $r = -0.90$, $P = 0.006$.

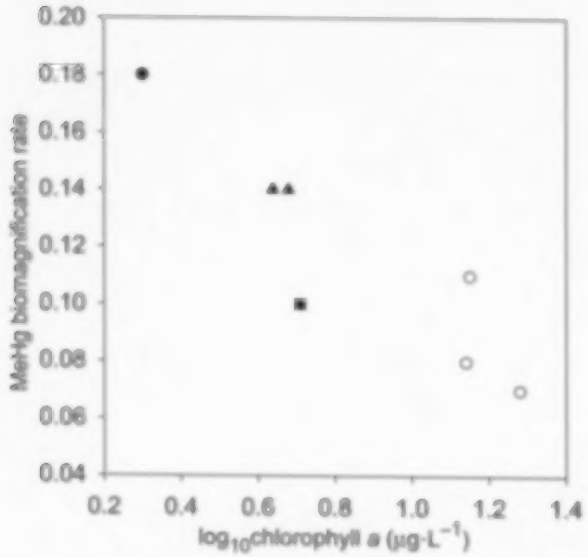
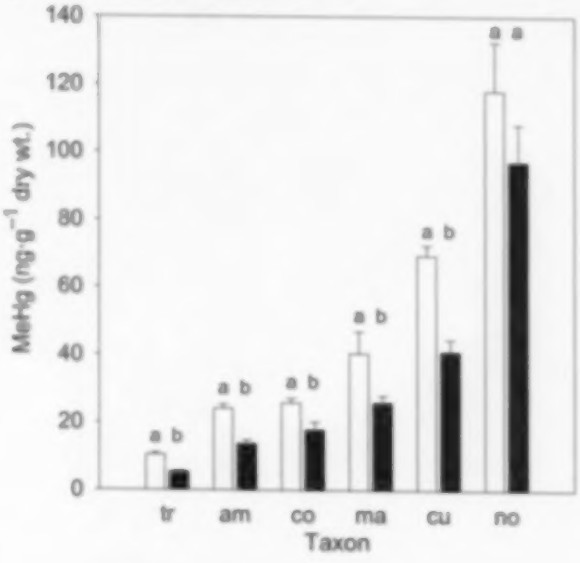


Fig. 7. Pre- and post-fire methyl mercury (MeHg) concentrations in biota from Delorme Lake. Letters indicate differences between pre- and post-fire periods (Student's *t* test, $P \leq 0.05$). Error bars represent +1 standard error of the mean.



test, $P = 0.05$). Slight post-fire increases were observed for alkalinity (22%) and DOC concentration (19%; $P = 0.05$ and 0.06). Most water quality parameters, including TP, chl *a*, and pH did not differ between pre- and post-fire years. However, TP and chl *a* concentrations were relatively high during the spring (~60 µg·L^{-1} and 20 µg·L^{-1}, respectively), and remained elevated through the summer, peaking during rain events.

Discussion

Influence of forest fire on MeHg

Two years after fire, MeHg concentrations in four of five macroinvertebrate taxa and in brook stickleback did not differ between burned and reference drainage basins in the Swan Hills. Our results are consistent with studies by Garcia and Carignan (1999, 2000) in Boreal Shield lakes, where MeHg concentrations in aquatic biota did not differ between burned and reference lakes. Garcia and Carignan (1999) attributed their results to high fire intensity and the volatilization of soil-bound Hg in the organic soil layer, which reduced the pool of organic carbon and Hg available for export. In the Swan Hills lakes, a positive correlation between colour and the ratio of DBA burned to lake volume (see Allen et al. 2003) suggested a short-term increase in the export of terrestrial humic substances to burned lakes; however, the inputs were likely insufficient to have a major effect on MeHg dynamics in the lakes.

Post-fire soil erosion and export of humic substances were likely limited by dry conditions during the study. The winter prior to the Swan Hills fire (1997–1998) was 30% drier than average (1942–2000). Precipitation and runoff in the first 2 years after the fire (1998 and 1999) were 25% and 33% lower, respectively, than the long-term average for the region (precipitation data from Alberta Sustainable Resource Development, PFFC Weather Section, 9920 – 108 Street, Edmonton, Alberta T5K 2M4, unpublished data; runoff data from Environment Canada 2002). Post-fire runoff in the Swan Hills (53–114 mm·year^{-1}, 1998–2000) was considerably lower than that reported from Boreal Shield catchments by Carignan et al. (2000; 500–700 mm·year^{-1}). Unlike the Boreal Shield, where high precipitation and thin soils promote surface runoff and erosion of denuded drainage basins, deep soils and glacial materials in Boreal Plain catchments likely act to reduce the potential for surface runoff. Moreover, several of the burned Swan Hills drainage basins had relatively low catchment to lake volume ratios, and burn impact ratios were likely too low to yield the runoff needed to have a major effect on lake water chemistry. Previous studies that have reported post-fire changes in lake water chemistry included drainage basins with larger DRs and higher percentage of disturbance than in the Swan Hills (e.g., Carignan et al. 2000; McEachern et al. 2000).

In contrast with our results from the Swan Hills fire, MeHg concentrations in Delorme Lake biota after the Chisholm fire decreased by 30%–50% relative to the pre-fire year. The decline in MeHg concentrations in Delorme Lake biota may have been related to post-fire inputs of DIN and (or) a subsequent increase in lake trophic status. Elevated concentrations of DIN in lake water have previously been associated with forest fire (McEachern et al. 2000; Carignan et al. 2000) and are attributed to leaching of mineralized N from soils (Tiedemann et al. 1978). Nutrient additions and increases in algal productivity have been shown to decrease MeHg concentrations in phytoplankton (Pickhardt et al.

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Table 4. Pearson's correlation coefficients for methyl mercury (MeHg) concentration in biota ($ng \cdot g^{-1}$ dry weight) presented with lake water chemistry, watershed characteristics, body size, and trophic position.

Taxon	Variable	*r*	*P*	*n*
Phryganeidae [MeHg]	arcsin CON (%)	0.74	**0.007**	10
	$\log_{10}$COND ($\mu S \cdot cm^{-1}$)	–0.68	0.02	
	$\log_{10}$Mg ($mg \cdot L^{-1}$)	–0.64	0.02	
	$\delta^{15}N_{adj}$ (‰)	0.88	**<0.001**	
Gammarus lacustris [MeHg]	arcsin CON (%)	0.87	**0.006**	7
	$\log_{10}K^+$ ($mg \cdot L^{-1}$)	–0.86	**0.007**	
	$\log_{10}$CHL *a* ($\mu g \cdot L^{-1}$)	–0.68	0.05	
	LGTH (mm)	–0.84	0.010	
	WT (mg, dry weight)	–0.71	0.040	
Cordulia shurtleffi $\log_{10}$[MeHg]	arcsin CON (%)	0.70	0.030	8
	$\log_{10}Ca^{2+}$	–0.88	**0.002**	
	$\log_{10}Mg^{2+}$	–0.90	**0.001**	
	TP ($\mu g \cdot L^{-1}$)	–0.88	**0.002**	
	pH	–0.94	**<0.001**	
	$\log_{10}$COND ($\mu S \cdot cm^{-1}$)	–0.74	0.02	
	$\log_{10}$CHL *a* ($\mu g \cdot L^{-1}$)	–0.72	0.02	
Aeshna spp. $\log_{10}$[MeHg]	WTLD (%)	0.87	**0.001**	8
	AW (%)	–0.78	**0.007**	
	DBA:LA	–0.78	**0.007**	
	pH	–0.81	**0.004**	
	TP ($\mu g \cdot L^{-1}$)	–0.85	**0.002**	
	$\log_{10}$Mg ($mg \cdot L^{-1}$)	–0.83	**0.003**	
	$\log_{10}$CHL *a* ($\mu g \cdot L^{-1}$)	–0.80	**0.005**	
Notonecta spp. [MeHg]	$\log_{10}$DOC ($mg \cdot L^{-1}$)	–0.50	0.05	12
	$\log_{10}$TDP ($\mu g \cdot L^{-1}$)	–0.64	0.01	
	Na^+ ($mg \cdot L^{-1}$)	–0.59	0.02	
Culaea inconstans $\log_{10}$[MeHg]	AW (%)	–0.83	**0.005**	8
	pH	–0.94	**<0.001**	
	ALK ($mg \cdot L^{-1}$)	–0.82	**0.006**	
	TP ($\mu g \cdot L^{-1}$)	–0.90	**0.001**	
	$\log_{10}$CHL *a* ($\mu g \cdot L^{-1}$)	–0.78	0.01	
	$\log_{10}Ca^{2+}$ ($mg \cdot L^{-1}$)	–0.85	**0.004**	
	$\log_{10}Mg^{2+}$ ($mg \cdot L^{-1}$)	–0.91	**0.001**	
	$\log_{10}$COND ($\mu S \cdot cm^{-1}$)	–0.72	0.02	

Note: CON, conifer cover; AW, aspen cover; WTLD, wetland cover; DBA:LA, drainage basin area : lake area; COND; conductivity; $\delta^{15}N_{adj}$, baseline-adjusted $\delta^{15}N$; ALK, alkalinity; TP, total phosphorus; TDP, total dissolved phosphorus; DOC, dissolved organic carbon; CHL *a*, chlorophyll *a*; LGTH, mean total length; WT, dry body weight; significant *P* values indicated in bold ($P \leq 0.007$).

2002) and fish (Essington and Houser 2003) via growth dilution, whereby an increase in the volume of algal cells dilutes the pool of bioavailable MeHg. Whereas late summer chl *a* and TP concentrations in Delorme Lake did not differ between pre- and post-disturbance years, there was some evidence of an increase in algal productivity during the early part of the summer immediately following the fire. Chl *a* concentrations were relatively high during this time ($20\ \mu g \cdot L^{-1}$), in contrast with the typical pattern exhibited by other lakes in the region, where chl *a* concentrations were $<5\ \mu g \cdot L^{-1}$ throughout June and July and then peaked in August (E.W. Allen, unpublished data). Increased primary productivity may have lead to increased growth rates of macroinvertebrates, as suggested by post-fire increases in body condition (weight:length) of Phryganeidae and *G. lacustris*. Increased growth rates may have contributed to a dilution of MeHg concentrations among primary and secondary consumers (Essington and Houser 2003).

The slightly elevated post-fire concentration of lake water DOC in Delorme Lake may also reflect an increase in lake productivity. Unlike in previous studies (e.g., McEachern et al. 2000), this increase was not accompanied by an increase in lake water colour, suggesting in-lake production of organic carbon. Inputs of allochthonous carbon were likely negligible in Delorme Lake because the organic layer was completely consumed by the fire, particularly in clear-cut portions of the catchment. Increased autochthonous DOC would be consistent with elevated algal production. Unlike inputs of allochthonous DOC, the production of autochthonous DOC is not associated with increased input of Hg, acidity, or colour, factors that appear to promote MeHg bioaccumulation or which are associated with high MeHg

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Table 5. Pre- and post-fire means (± standard error) for water chemistry in Deforme Lake.

Variable	Pre-fire (2000)	Post-fire (2001)	*P* value
Total phosphorus ($\mu g \cdot L^{-1}$)	47±2	47±2	0.65
Total dissolved phosphorus ($\mu g \cdot L^{-1}$)	11±0.9	10±0.6	0.18
Ammonium ($\mu g \cdot L^{-1}$)	7.2±1.4	75±23	0.05
Nitrate + nitrite ($\mu g \cdot L^{-1}$)	3.0±2.0	9.7±1.3	0.12
Dissolved organic carbon ($mg \cdot L^{-1}$)	32±0.1	38±0.3	0.06
Alkalinity (mg $CaCO_3 \cdot L^{-1}$)	122±0.5	144±2.3	0.05
pH	8.5	7.8	0.10
Colour (mg $Pt \cdot L^{-1}$)	71±0.4	69±4	0.56
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	25±3	21±2	0.44

Note: Pre-fire total and dissolved phosphorus and pH means were calculated from a single sample collected 4 August 2000, and two duplicate samples collected 30 August and 3 October 2000. All other pre-fire water chemistry means were calculated from two duplicate samples collected 30 August and 3 October 2000. Post-fire means were calculated from five duplicate samples collected between 16 August and 3 October 2001. *P* value calculated from Mann–Whitney *U* test.

concentrations in fish (e.g., Driscoll et al. 1995; Kelly et al. 2003). The effects of autochthonous DOC on MeHg dynamics are not clearly understood; however, like other organic compounds, it may readily bind Hg and MeHg in the water column and thereby reduce bioaccumulation.

Increases in lake trophic status and consequent declines in MeHg bioaccumulation may be more likely following forest fire than clear-cutting, assuming mineralization of organic soils during fire results in greater inputs of dissolved inorganic nitrogen and phosphorus than logging (Carignan et al. 2000; Pinel-Alloul et al. 2002). In the burned Foothills lakes, slightly elevated TP and chl *a* concentrations (1.3- and 1.5-fold, respectively) relative to reference Foothills lakes (Allen et al. 2003) may have had a mitigating effect on MeHg bioaccumulation immediately after the fire, as suggested by our Deforme Lake results. Unlike fire, logging leaves the organic layer intact, providing a source of DOC to lakes that in turn may shift water chemistry in favour of Hg methylation and uptake (e.g., Garcia and Carignan 2000). Post-disturbance export of DOC from boreal watersheds has been shown to be strongly dependent on watershed characteristics; burned peatlands increased the DOC concentrations of lakes studied by McEachern et al. (2000), whereas limited logging in Boreal Mixedwood lakes had no impact on lake DOC concentrations (Prepas et al. 2001). It remains unclear how logging would affect MeHg dynamics in the lakes of conifer-dominated drainage basins in the Boreal Foothills, where the potential for DOC export is higher than in Mixedwood catchments (Allen et al. 2003), and where relatively steep slopes and high precipitation may promote greater runoff and soil erosion than at lower elevations.

Influence of lake water chemistry on MeHg concentrations

Ecoregional variation in water chemistry appeared to influence MeHg concentrations in aquatic biota, which confounded comparisons of MeHg bioaccumulation between burned and reference drainage basins. MeHg concentrations in biota were negatively correlated with lake water pH, as well as with TP and dominant cation concentrations, all of which were higher in Mixedwood than in Foothills lakes. Similar relationships have been reported from other regions for zooplankton (Westcott and Kalff 1996) and larger fish species (Wren and MacCrimmon 1983; Grieb et al. 1990). The influence of pH on Hg methylation (Miskimmin et al. 1992) and MeHg bioavailability (Kelly et al. 2003), along with the influence of lake productivity on biodilution (Larsson et al. 1992), were likely the main factors that affected MeHg concentrations in biota. Poor relationships between MeHg in *Notonecta* and lake water chemistry may reflect their status as semi-aquatic insects capable of migrating from lake to lake (Clifford 1991). Positive correlations between MeHg concentration in primary consumers and conifer cover likely reflected the influence of water chemistry, since catchment vegetation covaried with many water chemistry variables (Allen et al. 2003). It is unclear whether trophic position or water chemistry had a greater influence on MeHg in Phryganeidae.

Allen et al. (2003) proposed that high productivity, pH, and water hardness in Mixedwood lakes was linked to their relatively low landscape position, which exposes the lakes to groundwater inputs from deep glacial tills rich in base cations. The dilute water chemistry of Foothills lakes at upper elevations reflects the diminished role of groundwater and the importance of precipitation in the water budget. The inclusion of Mixedwood lakes as reference lakes for the burned Foothills lakes likely obscured post-fire changes in nutrients, and may have exaggerated differences in MeHg concentrations between treatments. Thus, pre-existing water chemistry gradients, rather than a fire effect, likely explain high MeHg concentrations in *Cordulia shurtleffi* from burned lakes relative to reference lakes, since two of three reference lakes were in the Mixedwood region.

Unlike in earlier studies, MeHg concentrations in biota were not correlated with lake water DOC concentrations or colour. Positive relationships between lake DOC and Hg concentrations in zooplankton and fish are typically reported among lakes with low DOC concentrations (<10 $mg \cdot L^{-1}$), in which the DOC is primarily allochthonous (Driscoll et al. 1995; Garcia and Carignan 1999). Compared with other boreal regions, the Boreal Plain features highly productive lakes with high concentrations of autochthonous DOC (i.e., >30 $mg \cdot L^{-1}$), in addition to dystrophic lakes with high concentrations of allochthonous DOC. MeHg concentrations

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were not correlated with DOC or lake water color because the proportion of DOC from autochthonous and allochthonous sources was not consistent among lakes.

MeHg biomagnification

MeHg concentrations in the Swan Hills biota generally exhibited less biomagnification than reported in previous studies. The average slope of the $\log_{10}$MeHg versus $\delta^{15}N$ regression in Swan Hills lakes (0.15) was below the range of values determined for pelagic food webs from less productive systems (i.e., 0.2–0.3; Kidd et al. 1995; Bowles et al. 2001). On average, MeHg concentrations increased fivefold per trophic level in the Swan Hills food webs, compared with ~10- to 15-fold per trophic level in pelagic food webs (Plourde et al. 1997). The higher biomagnification rate in pelagic food webs may reflect the bioaccumulative MeHg burdens of longer-lived top predators (i.e., piscivorous fish). Littoral biota collected from the Swan Hills lakes all have relatively short lifecycles (i.e., <3 years; Clifford 1991); thus biomagnification is not enhanced by time of exposure to MeHg and bioaccumulative MeHg burdens.

Lower MeHg biomagnification rates in Boreal Plain lakes relative to those in other studies may also reflect higher lake trophic status, as suggested by negative correlations between MeHg biomagnification and lake productivity indices. These correlations may reflect a further dilution of MeHg in secondary consumers in productive lakes caused by higher growth rates. Previously, researchers have suggested that it is difficult to distinguish the effects of biodilution on MeHg concentrations in biota from those of water chemistry and bioavailability because productive lakes are characterized by conditions that are inhibitory to Hg and MeHg bioavailability, such as high pH, alkalinity, and base cation concentrations (Larsson et al. 1992; Kidd et al. 1999). Based on the results of fertilization experiments, Rudd and Turner (1983) suggested that elevated pH of lake water, and not biodilution, was the primary factor affecting Hg concentrations in nutrient-enriched systems. However, our results, as well as those from recent studies (e.g., Pickhardt et al. 2002), indicate otherwise. Because MeHg uptake among secondary consumers occurs primarily via prey consumption, the direct effects of lake chemistry on methylation and MeHg bioavailability cannot explain the different rates of MeHg biomagnification among lakes. Rather, we suggest that high growth rates among biota in productive lakes may dilute MeHg concentrations at each step in the food chain and cause an apparent decrease in biomagnification over multiple trophic levels.

Alberta Special Waste Treatment Centre lakes

MeHg concentrations in macroinvertebrates and brook stickleback from Edith Lake, located 10 km upwind of the ASWTC, were among the highest in the lake set, but were comparable with, or lower than, values reported for similar biota from remote Boreal Shield lakes with similar water chemistry (e.g., Hall et al. 1998; Kidd et al. 1999). Relationships between MeHg in biota and lake water pH indicated that MeHg concentrations in lakes near the ASWTC, though generally higher than in other lakes, were not higher than expected, based on their water chemistry. Most correlations between MeHg and environmental factors remained when the ASWTC lakes were removed, indicating that inclusion of these lakes did not skew the relationships between lake chemistry and MeHg concentrations in biota.

High MeHg concentrations in aquatic biota from these lakes appear to reflect lake water chemistry and other natural factors, rather than proximity to the ASWTC. For example, the Edith Lake drainage basin includes a relatively large bog (17% of DBA, highest among the 12 lakes) that is a probable source of colour, acidity, and MeHg for the lake (St. Louis et al. 1994). The relatively dilute water chemistry of Edith Lake and Chrystina Lake is a feature of their high landscape position, higher precipitation, and disconnect from groundwater inputs of base cations compared with lakes at lower elevations in the Mixedwood region (Allen et al. 2003). Moreover, it is unlikely that emissions from the ASWTC have had a major impact on water quality in either lake. In Chrystina Lake, situated 1.5 km from the ASWTC, lake water pH and other water quality parameters have not changed markedly during plant operations (e.g., pH = 6.8 and 7.1 in 1985 and 2000, respectively; Stanley and Associates Engineering Ltd. 1990). Also, as of 1990, emissions from the facility had not resulted in detectable increases in Hg deposition rates in the immediate vicinity of the ASWTC (Stanley and Associates Engineering Ltd. 1990). According to baseline-adjusted $\delta^{15}N$ values, biota in Edith and Chrystina lakes were trophically elevated relative to biota from burned and reference lakes, which may have further contributed to their elevated trophic MeHg. Thus high MeHg concentrations in the ASWTC lakes appeared to be attributable to drainage basin features, lake chemistry, and food web structure, rather than systemic contamination from emissions associated with hazardous waste disposal.

This is the first comprehensive study of MeHg dynamics and drainage-basin disturbance in aquatic food webs on the Boreal Plain. MeHg concentrations in aquatic biota were generally lower than reported from other boreal regions, due in part to the higher pH and trophic status of Boreal Plain lakes. Unlike in other regions, DOC concentration was not a strong predictor of MeHg in aquatic biota due to high concentrations of autochthonous DOC in productive lakes. Post-fire inputs of terrestrial DOC in Boreal Plain lakes might therefore be better gauged by changes in lake colour or light extinction rather than by DOC concentration. The influence of forest fire on MeHg dynamics will largely depend on watershed characteristics, the extent of damage to the soil organic layer, and changes in lake-water chemistry. Based on a few studies in a wide range of catchment types, the short-term effects of forest fire on the Boreal Plain include increased DOC concentrations, transient eutrophication, and a shift in the algal community towards cyanobacteria (McEachern et al. 2000; Allen et al. 2003). Where forest fire causes an increase in primary productivity, MeHg concentrations in aquatic biota may decrease temporarily as a result of biodilution.

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Effects of turbidity on the reactive distance, search time, and foraging success of juvenile Atlantic cod (*Gadus morhua*)

Justin J. Meager, Turid Solbakken, Anne C. Utne-Palm, and Tina Oen

Abstract: We investigated the effects of turbidity on the foraging behaviour of juvenile Atlantic cod (*Gadus morhua*) on mysid prey (*Praunus neglectus*) in the laboratory. The influence of turbidity on vision and chemoreception was examined by measuring reactive distances and search times to visual, chemical, and visual–chemical prey cues over turbidity levels ranging from 0.4 to 17·m^{-1} (beam attenuation·m^{-1}). We also compared foraging rates of juvenile cod on mysids in highly turbid water and clear water under well lit and totally dark conditions. Juvenile cod using chemical cues were able to locate mysids from significantly longer distances than when only visual cues were available. Turbidity did not affect reactive distance to chemical cues, and had only a weak negative effect on reactive distance to visual and visual–chemical cues. Search time was variable, but tended to increase with turbidity. Turbidity did not affect predation rates on free-ranging mysids, but predation rates were significantly lower in dark conditions than in well lit conditions. We suggest that juvenile cod use chemoreception in conjunction with vision (at close ranges) to locate prey in highly turbid water.

Résumé : Nous avons étudié les effets de la turbidité sur le comportement de prédation de jeunes morues franches (*Gadus morhua*) sur des proies mysidacées (*Praunus neglectus*) en laboratoire. Nous avons déterminé l'influence de la turbidité sur la vision et la chimioréception en mesurant les distances de réaction et la durée de la recherche en fonction de signaux visuels, chimiques et chimiques–visuels des proies sur une gamme de turbidités de 0.4 à 17·m^{-1} (atténuation du faisceau·m^{-1}). Nous avons aussi comparé les taux d'alimentation des jeunes morues sur les mysidacées dans de l'eau fortement turbide et de l'eau claire dans des conditions de bonne lumière et d'obscurité totale. Les jeunes morues sont capables de localiser les mysidacées à l'aide de signaux chimiques à des distances significativement plus grandes qu'à l'aide de seuls signaux visuels. La turbidité n'affecte pas la distance de réaction aux signaux chimiques et elle n'a qu'un faible effet négatif sur la distance de réaction aux signaux visuels et chimiques–visuels. La durée de la recherche des proies est variable, mais elle tend à augmenter en fonction de la turbidité. La turbidité n'affecte pas les taux de prédation sur les mysidacées libres, mais les taux de prédation sont significativement plus bas en conditions d'obscurité que de bon éclairement. Nous pensons que les jeunes morues utilisent la chimioréception en conjonction avec la vision (à courte distance) pour localiser leurs proies dans les eaux très troubles.

[Traduit par la Rédaction]

Introduction

Growth and survival of juvenile Atlantic cod (*Gadus morhua*) depends on their ability to detect and capture prey items, such as small crustaceans and fish (Hawkins et al. 1985; Grant and Brown 1998), which are often highly mobile and difficult to catch (Arnott et al. 1998; Arnott and Pihl 2000). The ability of fish to visually detect such prey can be limited by prey visibility and the optical environment (e.g., spectral composition, light intensity, turbidity; Vinyard and O'Brien 1976; Utne-Palm 1999). Turbidity from suspended sediment, dissolved organic matter, and plankton scatters and absorbs light and can reduce the reactive distance of fish (Gregory and Northcote 1993; Vogel and Beauchamp 1999 and references herein).

Coastal habitats used as nurseries by juvenile cod (Godø et al. 1989; Grant and Brown 1998) may be affected by increases in turbidity from human activities that lead to eutrophication and suspension of sediments (Bonsdorff et al. 1997; Frid et al. 2003). However, the influence of turbidity on the foraging success of juvenile cod is unknown.

Turbidity can have varying effects on foraging by fishes depending on their search volume and foraging mode. For example, an intermediate increase in turbidity can have a positive effect on feeding rates of fish and fish larvae feeding on small prey by increasing contrast (Boehlert and Morgan 1985; Utne 1997). Predators that locate prey with nonvisual senses, such as chemoreception or mechanoauditory reception, may be unaffected by turbidity (Rowe et al. 2003). Although vision is considered to be the main sense used by juvenile cod to detect mobile prey (Brawn 1969; Chinarina and Troshicheva 1975), chemosensory cues are also used (Harvey and Batty 1998; Løkkeborg 1998;

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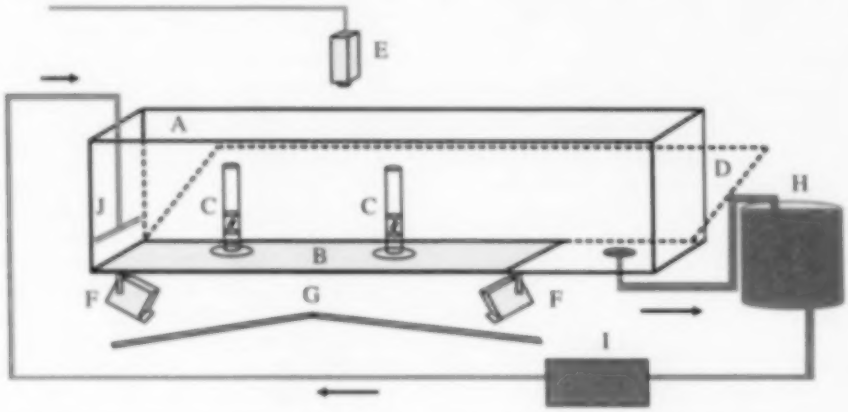
Fig. 1. Experimental set-up: glass aquarium (A; 300 cm × 70 cm × 50 cm); experimental arena (B; 200 cm × 70 cm × 30 cm); polyvinyl chloride prey cylinders (C; diameter, 7 cm) containing a mysid with vertical movement restricted by two clear disks; mirror providing side view (D), a 5-cm grid was painted on the back wall of the aquarium; overhead video camera (E; Panasonic WV BP550) with infrared filter (Optolite 50% IR); infrared lamps (F; 2 × Derwent 70 w, 830 nm); reflective white boards (G); tank (H) where saltwater and kaolin were mixed by air bubbling and circulation; pump (I; 58 L·m^{-1}) introducing turbid water mix into aquarium through jets (J).



Løkkeborg and Fernö 1999) and may be important in highly turbid water.

Our experiments aimed to examine the influence of turbidity on the reactive distance and search time of juvenile cod, and to determine if turbidity affects their foraging rates. We also tested the general hypothesis that cod mainly use visual cues to locate prey in clear water (in photopic conditions) and chemoreception in turbid water.

Materials and methods

Collection and maintenance of experimental animals

Juvenile *Gadus morhua* were collected at several sites around Bergen, western Norway (60°16′N; 4°58′E) using trammel nets. These fish were tagged with external Floy T-bar tags and kept in tanks (1000 L) for up to 3 months prior to the experiments. Juvenile cod were fed a diet of frozen gobies, decapods, pellets, and live mysids (the use of live decapods and fishes as prey items is prohibited by the Norwegian Council for Animal Research).

Mysids (*Praunus neglectus*) were used for experiments because they are common prey items of juvenile cod (e.g., Mattson 1990; Hüssy et al. 1997) and have an evasive response similar to other natantid prey of juvenile cod (tail-flip response; Neil and Ansell 1995). *Praunus neglectus* were collected using dip and seine net. Mysids were kept in tanks (90 L) for up to 2 months prior to the experiments and fed live artemia.

Experiment 1: effect of turbidity on the reaction distance and search time of juvenile cod

In this experiment we looked at the influence of turbidity on prey localization by juvenile cod. Mysids were located inside closed (clear) or partially closed cylinders (clear and white) to provide cod with visual, visual–chemical, and chemical prey cues.

Experimental set-up

Experiments were conducted in a large rectangular glass aquarium (70 cm × 300 cm × 50 cm), filled to a depth of 20 cm with seawater (salinity 32‰–35‰; temperature maintained at 9.5 ± 1.5 °C). This depth allowed us to view the fish from above at the highest turbidity levels.

Diffuse light conditions were provided (9.5 ± 0.5 μmol·m^{-2}·s^{-1}) by indirectly illuminating the aquarium with four halogen floodlights (2 × 150 W, 2 × 500 W). This irradiance level is equivalent to that found in juvenile cod habitat in coastal waters of western Norway (i.e., at a 20-m depth on a sunny clear summer day with 1000 μmol·m^{-2}·s^{-1} surface irradiance, or at a 3-m depth on a cloudy winter day with 20 μmol·m^{-2}·s^{-1} surface irradiance; Baliño and Aksnes 1993). The experimental aquarium was illuminated from underneath with disperse infrared light (>800 nm), and fish silhouettes were recorded using an overhead video camera fitted with an infrared filter (Fig. 1).

Pulverised kaolinite (Kaolin Polsperse 10, ECC International) was used to make the water turbid. A kaolin–seawater suspension was introduced into a mixing tank and recirculated through the experimental tank (Fig. 1). Water flow was turned off 30 min prior to the experiments.

Experimental protocol

We examined the reactive distance and search time of juvenile cod in response to three prey treatments (visual, visual–chemosensory, and chemosensory cues) at five turbidity levels (beam attenuation: 0.4, 3, 5, 10, and 17·m^{-1}). Prey treatments consisted of mysids in clear cylinders, (visual), mysids in clear cylinders with small holes (visual–chemical), and mysids in white cylinders with small holes (chemical). Additional empty cylinders (white, clear, and with holes) were used as controls for the effect of cylinders on reactive distance and search time. The size (2.5 mm diameter), number, and location (above and below the section containing the mysid) of holes were chosen to allow chemical cues to disperse from the cylinders while minimizing

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mechano-auditory cues. Observations of dye tracers showed that chemical cues dispersed uniformly around each cylinder. The depth of mysids (from 7.5 to 12.5 cm) was constant throughout all trials (Fig. 1).

The turbidity levels correspond to 1.4, 10, 16, 32, and 55 nephelometric turbidity units (NTU; measured with a Vernier turbidity sensor) and represent the turbidity range in habitats used by juvenile Atlantic cod (based on turbidity data from E. Marken, Department of Physics and Technology, University of Bergen, N-5020 Bergen, Norway; McMahon et al. 1992; Bowers et al. 2000; Frette et al. 2004).

Turbidity levels were maintained to within ±0.5 beam attenuation·m^{-1} throughout the experiments. Water samples from random locations at both the top and bottom of the tank at the start and finish of each trial were used to measure turbidity during a trial. Turbidity was measured as the percentage of light transmitted through a 10-cm cuvette in a spectrophotometer (Shimadzu UV-VIS Recording Spectrophotometer UV-160) at 800 nm (to minimize near-forward scattering) and converted to beam attenuation using the standard relationship $T = e^{-cr}$, where T is light transmitted through a path length r (in metres) and c is the beam attenuation coefficient.

A total of 18 fish (20–30 cm standard length, SL) were tested for each turbidity–prey cue treatment. Fish were tested in pairs following the randomized-blocks experimental design: nine pairs of fish received each treatment once in different orders. To maintain equal replication in each treatment (18 fish), fish that did not complete every treatment combination were replaced with new individuals (three fish grew too large, one died, and another seven developed exophthalmia).

Fish were starved for between 4 and 5 days (to increase feeding motivation; e.g., Confer et al. 1978) prior to the experiments, and acclimated overnight to the experimental tank. Trials lasted for 1.5 h or until each fish reacted to prey at least three times. Every 3 weeks throughout the experiments, the fish were anaesthetised (Metacaine) and eye size (diameter, mm), standard length (cm), and weight (g, wet weight) were measured.

Mysids (14–23 mm total length, TL) were acclimated to the experimental room in 200 mL of water from the experimental aquarium (one mysid per 200 mL) for 30–45 min before experiments. Mysids were subsequently introduced into the prey cylinders at the start of each trial, when fish were general facing away from the cylinders and at the opposing end of the tank. Two cylinders were placed in the experimental tank in each trial; one cylinder was placed randomly in each half of the experimental area.

Search time and reactive distances were determined by analyzing video recordings. Reactive distance was defined as the distance at which fish first orient toward prey (Vinyard and O'Brien 1976; Vogel and Beauchamp 1999). This behaviour was easily distinguished from more random and slower search activity by fish; initiation of pursuit was accompanied by direct orientation of the long axis of the fish towards the prey. Reactions of cod to prey were digitized, and distances were measured using Image-Pro Plus (Version 5, Media Cybernetics Inc., Silver Spring, Maryland). The maximum reactive distance of the first three observations for each fish was used to represent reactive distance (Utne 1997; Utne-Palm 1999). A side mirror and 5-cm grid (Fig. 1) were used to determine the vertical location of cod (where possible). We determined search time by recording the time from when mysids were introduced into the cylinders to the first reaction for each fish.

Data analysis

Data were analysed using SPSS (Release 11.5, SPSS Inc. 1999). The effect of turbidity and prey cues on the responsiveness of cod to prey was tested with logistic regression. The goodness of fit of a particular model was determined using the Likelihood Ratio statistic (G^2; Sokal and Rohlf 1995). We used a χ^2 test to test for an association between reactive depth category (5–10 cm, 10–15 cm, and 15–20 cm) and turbidity.

Factorial, random-blocks analysis of variance (ANOVA) was used to test for the differences in reactive distance and search time between turbidity levels and prey cues. Both individual fish and pairs of fish were used as blocks in the analysis to remove variability associated with individuals or interactions between pairs. Before analysis, reactive distance and search time were tested for normality and homoscedasticity using the Shapiro–Wilk and Levene's tests, respectively. No transformation was necessary for reactive distance, but search times were $\log_{10}$-transformed. Tukey's test was used for post hoc comparisons of means.

As any differences in turbidity within each category would increase the variability within the treatments in the ANOVAs, relationships between turbidity and reactive distance, as well as between turbidity and search time, were also investigated using a linear regression approach. We used multiple regression to determine whether turbidity, fish size (SL and weight), fish eye size, and mysid size (TL) significantly affected reactive distance and search time ($\log_{10}$-transformed). The forward stepwise model was used, and the standardized residuals were examined to test for homoscedasticity (Sokal and Rohlf 1995). The turbidity variable was the $\log_e$-transformed mean of measurements taken at the start and finish of each trial.

Experiment 2: effect of turbidity on the foraging rate of juvenile cod

We aimed to test if an ecologically relevant maximum turbidity (upper estuary; e.g., McMahon et al. 1992) affected foraging rates of juvenile cod on free-ranging mysids.

Experimental set-up

Experiments were conducted in a large, rectangular glass aquarium (70 cm × 300 cm × 50 cm), filled to 35 cm with seawater (Fig. 1). As in all laboratory experimental studies, enclosure effects were unavoidable. These experiments were therefore not intended to represent actual foraging rates and search times in nature but rather the relative changes in feeding rate and prey detection in response to changes in turbidity.

Experimental protocol

Predation rates on free-swimming mysids were compared between clear (c = 0.2·m^{-1}, 0.8 NTU) and highly turbid water (c = 28·m^{-1}, 91 NTU). We also measured predation rates

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in the complete absence of light (a sealed, dark room and clear water: c = 0.2·m^{-1}, 0.8 NTU) to determine if visual cues were used to locate and capture mysids. Cod were tested individually to determine foraging rates for each fish, with nine different fish tested in each treatment (clear, photopic; turbid, photopic; clear, no light). We used the same protocol as we did in the previous experiment to establish and measure turbidity levels.

Prior to each trial, one juvenile cod (20–30 cm SL) was starved for 48 h and acclimated to the experimental tank for 24 h. Ten mysids (14–23 mm TL) were acclimated to an adjacent glass aquarium that was maintained at the same turbidity level as that in the experimental tank using a recirculating pump system. At the start of experiments, mysids were released into the opposing end of the tank from the fish. The length and weight of mysids was measured prior to each experiment. After 2 h, the fish was removed, the tank was completely drained through a filter, and the remaining mysids were counted, weighed, and measured (TL, mm). The predation rate was measured as both the number of mysids consumed per h and the proportion of total mysid weight consumed.

Data analysis

Analysis of covariance (ANCOVA) was used to test for differences in predation rate (mysids eaten·h^{-1}) and proportion of mysid weight consumed (arcsine-transformed) between turbidity and light levels (turbid, clear, and dark), after variance associated with cod weight was removed. The homogeneity of regression coefficients in ANCOVAs was tested by examining the interaction between the covariate and factors (Dunn and Clark 1987; SPSS Inc. 1999).

Results

Experiment 1: effect of turbidity on the reaction distance and search time of juvenile cod

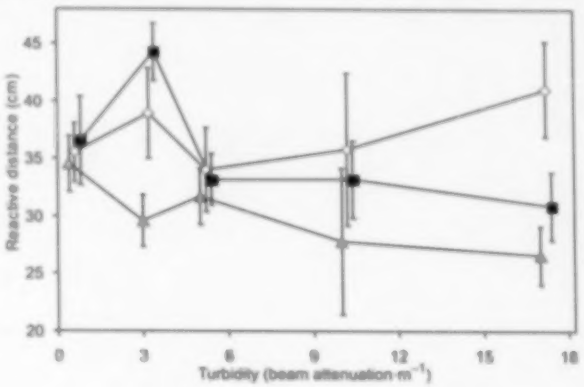
Approximately half of the 18 fish tested responded to prey in each treatment. The probability of cod responding to prey was not significantly affected by turbidity, prey cues, or interactions between turbidity and prey cues (logistic regression: turbidity, p = 0.30; prey cue, p = 0.64; interaction term, p = 0.63). Since no attacks were recorded on empty cylinders (from 84 observations), further analyses on reactive distances excluded trials with empty cylinders.

Most fish (87%) responded to prey at depths between 10 and 15 cm (112 reactions to prey were visible in the side mirror from a total of 355 reactions). Reactive depth was not significantly associated with turbidity (χ^2_8 = 11.7, p = 0.16). Consequently, we ignored depth-related changes in fish position in our analyses of reactive distance.

Effect of turbidity and prey cue on the reactive distance of juvenile cod to prey

The mean reactive distances to chemical cues (mean ± 1 standard error (SE), 36.9 ± 1.8 cm) and visual–chemical cues (35.4 ± 1.4 cm) were similar (Tukey's test, $p > 0.05$), and longer than to visual cues (29.9 ± 1.3 cm; Tukey's test, $p < 0.05$; ANOVA, $F_{[2,98]}$ = 7.42, p = 0.001). There was no significant effect of turbidity on cod reactive distance (ANOVA, $F_{[4,98]}$ = 0.32, p = 0.87), but there was a near-significant interaction between turbidity and prey cue (ANOVA, $F_{[8,98]}$ = 1.76, p = 0.094). Reaction distances to visual cues tended to decrease with turbidity, while reactive distances to visual–chemical cues tended to decrease after an initial increase (Fig. 2). Reactive distances to chemical cues tended to increase with turbidity (Fig. 2).

Fig. 2. Reactive distance (mean ± 1 standard error) of juvenile *Gadus morhua* to visual (shaded triangles), visual–chemical (solid squares), and chemical cues (open diamonds) with increasing turbidity. Data were offset slightly horizontally to display standard error bars.



We further investigated the relationship between reactive distance and turbidity with multiple linear regression. Turbidity reduced reactive distance to visual (r^2 = 0.09; df = 1,45; p = 0.044) and visual–chemical prey cues (r^2 = 0.08; df = 1,50; p = 0.049), but not to chemical prey cues (r^2 = 0.01; df = 1,40; p = 0.466). However, the relationship between turbidity and reactive distance was very weak and explained only 7.6% of variation for visual–chemical cues and 8.7% of variation for visual cues. Fish size, fish eye size, and mysid size did not contribute significantly to any of the regression models.

Effect of turbidity and prey cue on the search time of juvenile cod for prey

Although highly variable, search time tended to increase with turbidity (Fig. 3). There were no significant differences in mean search times between turbidity levels, prey cues, or interactions (ANOVA: turbidity, $F_{[4,75]}$ = 0.72, p = 0.58; prey cues, $F_{[2,74]}$ = 0.35, p = 0.71; interaction term, $F_{[8,74]}$ = 0.68, p = 0.71). Further analysis with multiple linear regression revealed a weak but significant increase in search time with turbidity for chemical (r^2 = 0.12; df = 1,36; p = 0.037) and visual–chemical prey cues (r^2 = 0.18; df = 1,48; p = 0.009; Fig. 3). Turbidity did not significantly affect search time for visual prey cues (r^2 = 0.01; df = 1,39; p = 0.54), although predator length had a weak positive effect on search time (r^2 = 0.18; df = 1,39; p = 0.006). Fish size, fish eye size, and mysid size did not contribute significantly to regression models for chemical or visual–chemical prey cues.

Experiment 2: Effect of turbidity on the foraging rate of juvenile cod

Mean predation rates (mysids·h^{-1}) did not differ significantly between highly turbid (mean ± 1 SE, 1.9 ± 0.6 mysids·h^{-1}) and

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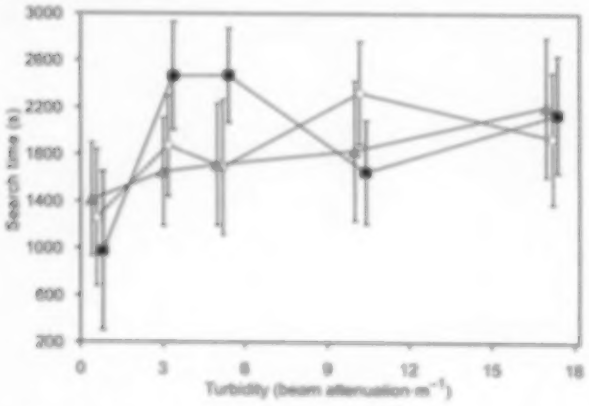
Fig. 3. Search time to reaction (mean ± 1 standard error) of juvenile *Gadus morhua* to visual (shaded triangles), visual–chemical (solid squares), and chemical cues (open diamonds) with increasing turbidity. Data were offset slightly horizontally to display standard error bars.



clear water (1.9 ± 0.6 mysids·h^{-1}), but were lower in complete darkness (0.79 ± 0.3 mysids·h^{-1}) (ANCOVA, $F_{[2,23]} = 4.25$, $p = 0.03$; Tukey's test, $p < 0.05$; Fig. 4). The same pattern was evident when predation rates were expressed as the proportion of mysid weight eaten (ANCOVA, $F_{[2,23]} = 8.80$, $p = 0.007$; Tukey's test, $p < 0.05$).

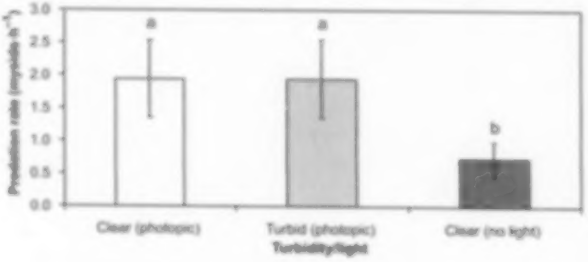
Discussion

Our results suggest that juvenile cod use vision and chemoreception to locate mysids in both clear and turbid water. Turbidity had little influence on the ability of cod to locate stationary mysids or to consume free-ranging mysids.

The lower predation rates in complete darkness and the weak effect of turbidity on visual reactive distance indicate that vision was used in highly turbid water. This reduced predation rate in the dark was unlikely to be caused by diel differences in feeding motivation, because all experiments were conducted at approximately the same time. Furthermore, numerous studies have indicated that diel foraging patterns of juvenile cod (> age 1) are variable, depending on prey availability (e.g., Turuk 1973; Mattson 1990; Gibson and Robb 1996).

Juvenile cod were able to accurately localize prey from longer distances using chemical cues rather than visual prey cues. Chemosensory response distance varies with the release rate and chemical composition of the prey odour, and with the fluid dynamic conditions in the habitat (Finelli et al. 2000). In the field, cod have been shown to respond to bait within a simple current from substantial distances outside visual range through rheotaxis (up to 698 m; Løkkeborg 1998; Løkkeborg and Fernö 1999). The present study shows that juvenile cod can use chemoreception to locate small, intact prey from outside visual range, even when water flow is limited to that created by fish movements (observations on dye tracers; J.J. Meager, unpublished data). However, the reliance of juvenile cod on chemoreception may be diminished in turbulent habitats or on other prey items (Weissburg et al. 2002).

Fig. 4. Foraging success of *Gadus morhua*, predation rate (mean mysids eaten·h^{-1} ±1 standard error), with turbidity and light (clear, $c = 0.2$ m^{-1}; turbid, $c = 28$ m^{-1}). Also shown are the results of Tukey's tests (a, homogenous subset 1; b, homogenous subset 2).



The longer reactive distances, and similar search times in response to chemical prey cues when compared with visual cues, indicates that chemoreception alone can be sufficient for foraging on stationary or slow moving prey items, such as polychaetes or bivalves (Mattson 1990; Hüssy et al. 1997). However, foraging rates of juvenile cod on free-ranging mysids were reduced in the absence of visual cues. Observations under infrared lighting suggest that this is a result of reduced attack success and not a lower encounter rate (J. Meager, unpublished data). Chemical cues last beyond the moment of production and may be less effective in the final stages of localizing and capturing rapidly moving or evasive prey (New et al. 2001; Brown 2003). The demands of these prey encounters are such that both visual and mechano-auditory information are likely to be required (New et al. 2001; Montgomery et al. 2002). A combination of distance chemoreception, vision at closer ranges, and possibly mechano-auditory reception may be an effective foraging strategy for juvenile cod to use with evasive prey items in well lit, highly turbid water.

It is also important to consider the influence of turbidity on the ability of prey to detect and avoid predators (Giske et al. 1994). In other studies, the absence of turbidity effects on predation has been attributed to compensatory factors acting on the reduced visual ability of predators, such as increased prey encounter or reduced antipredator behaviour of prey (Granqvist and Mattila 2004). Although littoral mysids are more active at lower light levels (Mauchline 1980; Viherluoto and Viitasalo 2001), the influence of turbidity on encounter rates between cod and mysids is unknown. Turbidity may also affect more the ability of mysids to see and evade the cod predator than the ability of cod to see mysids because cod present a larger visual target (Giske et al. 1994; de Robertis et al. 2003). The visual system of fishes also differs fundamentally from that of crustaceans; hence, mysid vision may not be affected by turbidity in the same way as their predators.

Juvenile cod had long reactive distances to visual cues in highly turbid water (mean ± 1 SE, 26.6 ± 2.6 cm at $c = 17$·m^{-1} for 800 nm, or 61 NTU), supporting observations that cod have a well developed visual system (Chinarina and Troshicheva 1975) and high visual acuity (Anthony 1981). The exact nature of the relationship between turbidity and visual reactive distance was difficult to determine in the current study because of the high variation in reactive distance.

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As fish and prey sizes, temperature, acclimation, and starvation time were all controlled for, this was attributed to differences in feeding motivation between individuals. Variation in movement, transparency, and position of mysids in the cylinders may have also affected reactive distance (Aksnes and Giske 1993; Thetmeyer and Kils 1995; Utne-Palm 1999). However, initial observations suggested that mysids were inactive and perched on one side of the prey cylinders.

The importance of vision in prey localization will obviously decrease at higher turbidity levels ($c > 28 \cdot m^{-1}$) and lower ambient light levels (e.g., at night or during arctic winters; dos Santos and Falk-Petersen 1989) than were used in this study. Juvenile cod in coastal waters of western Norway are unlikely to encounter turbidity levels greater than $5 \cdot m^{-1}$ (c; Godø et al. 1989; Frette et al. 2004; E. Marken, Department of Physics and Technology, University of Bergen, N-5020 Bergen, Norway, unpublished data). Elsewhere, turbidity levels of $20 \cdot m^{-1}$ (c) or above would only be encountered by cod in upper regions of highly turbid estuaries (McMahon et al. 1992; Kocum et al. 2002, based on estimates of c from diffuse attenuation coefficients K following Aksnes and Giske 1993).

The ability of juvenile cod to locate and capture a variety of prey items using a combination of sensory systems (e.g., Mattson 1990) explains, in part, how this species is able to exploit habitats ranging from clear coastal waters (Godø et al. 1989; Grant and Brown 1998) to turbid estuaries (Riley and Parnell 1984; Marshall and Elliot 1998). Although it has been suggested that cod may prey on mobile prey items when visibility is high and on benthic prey items at low visibility (Løkkeborg 1998; Løkkeborg and Fernö 1999), little is known of how the optical environment effects prey choice.

In summary, juvenile cod were able to locate prey in highly turbid water using a combination of vision and chemoreception (and possibly other senses). Hence, turbidity had little effect on the ability of juvenile cod to locate and consume mysids. Predicting the effects of turbidity on foraging by juvenile cod in the field will require further experiments on other prey items, such as fishes and decapods. Fully resolving the effects of turbidity on predation by cod on mobile prey will require an integrative approach, considering encounter rates and reactive distances from the perspective of both predator and prey.

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Exploratory assessment of historical recruitment patterns using relative abundance and catch data

Carl J. Walters and Ray Hilborn

Abstract: Historical apparent recruitment rates can be estimated from time series of relative abundance and catch data using a simple difference equation in conjunction with assumed catchability coefficient and natural survival rate. Simple smoothing and optimal linear state estimation procedures can remove much of the variance in such estimates due to measurement error in relative abundance. The apparent patterns can be helpful to reveal important recruitment changes prior to availability of data for more complex stock assessments, provide checks on recruitments estimated by more complex assessment procedures, and provide warnings about biases in other assessment procedures that might result from using the relative abundance data for model tuning.

Résumé : Des séries chronologiques de l'abondance relative et des données de capture permettent d'estimer les taux de recrutements apparents du passé lorsqu'on utilise une simple équation de différence conjointement avec le coefficient de capturabilité assumée et le taux naturel de survie. Un lissage simple et des procédures d'estimation de l'ordre linéaire optimal peuvent éliminer de ces estimations une grande partie de la variance due aux erreurs de mesure de l'abondance relative. Les patterns apparents peuvent servir à révéler des changements importants de recrutement avant que des données ne soient disponibles pour faire des évaluations plus complexes du stock; ils permettent de faire des vérifications des recrutements estimés par des méthodes plus complexes d'estimation; ils fournissent enfin des mises en garde concernant les erreurs qui peuvent survenir dans les autres méthodes d'évaluation qui utilisent les données d'abondance relative pour l'ajustement des modèles.

[Traduit par la Rédaction]

Introduction

Reconstruction of historical changes in recruitment rates has become a central concern of fisheries stock assessment, with recognition of the need to provide information on possible impacts of regime changes, recruitment overfishing, and trophic interaction effects that are likely to be concentrated on juvenile fish. Most reconstructions are now based on analysis of age–size composition data, with simpler relative abundance information relegated to a "tuning" role in the assessment calculations. Often, this leads to a shifting baseline problem, where composition data are available for only relatively recent years, and to inappropriate constraints on the recruitment estimates through assumptions about form and stationarity of stock–recruitment relationships.

This paper suggests that we should attempt to recover information on recruitment trends from simple relative abundance (spatial catch per unit effort, survey) data whenever possible using a method very similar to the time series differencing methods proposed by Schaefer (1954, 1957) for estimating surplus production. Schaefer noted that if we have a relative abundance series y_t that we are willing to assume was proportional to biomass B_t, i.e., $y_t = qB_t$, and if $B_{t+1} = B_t + P_t - C_t$, where P is production and C is catch, then an estimate of production for each year would be just $P_t = y_{t+1}/q - y_t/q + C_t$ (estimate B_t as y_t/q, where q is catchability, and solve biomass change equation for the production term). Schaefer's suggestion has not been pursued in recent years by workers other than Hilborn (2001) because of obvious problems with high variance in the P_t estimates caused by noise in the y_t data and because of concern about biases owing to changes in q (temporal trends owing to technological changes and targeting practices and density dependence in q in the form of hyperstability or hyperdepletion). We argue below that it is possible to at least partly surmount the first of these problems (variance in y_t) by using data smoothing and state estimation techniques.

Recruitment estimates as catch plus weighted change in abundance

Suppose we have a time series of numerical relative abundance data y_t, converted if necessary from biomass to numbers units by dividing the biomass data by estimates of average weight of fish and corrected insofar as possible for changes in q by using methods such as spatial averaging of

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raw catch rate data (e.g., Walters 2003). Suppose that we are willing to assume that numbers N_t of fish available to the relative abundance indexing method have changed according to the simple balance relationship

$$(1) \qquad N_t = S(N_{t-1} - C_{t-1}) + R_t$$

where $S = e^{-M}$ is a constant (and assumed known) natural survival rate, C_t is numbers catch, and R_t is recruitment. This is similar to the balance relationship assumed by Kirkwood et al. (2001) in their CEDA software for catch-effort analysis. Note that R_t will typically consist of a mixture of fish of different calendar ages (typically fast-growing individuals from more recent cohorts and slower-growing individuals from earlier cohorts); hence, it does not typically represent a particular birth year cohort. Further, R_t does not include, and may be impacted by, catches of younger fish than those included in the fishery that took C_t; for example, R_t to longline tuna fisheries consists of older fish that have been impacted at younger ages in recent years by purse-seine fisheries.

Then, assuming $y_t = qN_t$ for data years $t = 1,..., T$ and estimating N_t from an assumed q as $\hat{N}_t = y_t/q$, the "Schaefer" estimate of recruitment for each year $t > 1$ would be simply

$$(2a) \qquad R_t = y_t/q - S(y_{t-1}/q - C_t) = y_t/q - Sy_{t-1}/q + SC_t$$

or more generally

$$(2b) \qquad R_t = \hat{N}_t - S(\hat{N}_{t-1} - C_t)$$

where $\hat{N}_t$ is any estimate of N_t based on the relative abundance data. Note that this estimate has two components, one from change in relative abundance and one from the catch, differing from Schaefer's production estimates only in the use of numerical data and in correction for natural mortality effects through S. Further, it suffers from exactly the same variance pathology as Schaefer's production estimates. If y_t has independent measurement process errors v_t ($y_t = qN_t + v_t$) with variance σ_v^2, then the estimates of R_t from eq. 2 have an artificial variance component $\sigma_v^2(1 + S^2)/q^2$, which can easily mask any real trends or patterns in R_t even if the coefficient of variation (CV) of y is quite low (0.05 or more).

For those who are uncomfortable with the discrete time balance relationship eq. 1, recruitment and natural mortalities can be viewed as continuous variables using the differential equation $dN/dt = R_t - C_t - MN_t$. If R_t and C_t (as rates in numbers of fish per time) are treated as piecewise constant over intervals of one time unit, the solution of this equation is $N_t = (R_t - C_t)/M + [N_{t-1} - (R_t - C_t)/M]e^{-M}$, which implies recruitment estimates $[\hat{N}_t - \hat{N}_{t-1}e^{-M}]M/(1 - e^{-M}) + C_t$ corresponding to eq. 2*b*. Typically, the estimates from this continuous version of eq. 2 are larger than predicted by 2*b* but have essentially the same time trends (one component from C_t and another from changes in N).

Options for smoothing relative abundance data

There are cases where direct application of eq. 2 appears to give very reasonable estimates of recruitment patterns given reasonable estimates of q, for example, the Japanese longline data for tunas and billfish. But in most cases, eq. 2 gives R_t estimates that are unrealistically variable and (or) have at least some physically impossible values ($R_t < 0$).

For exploratory analysis of recruitment trends, it is generally necessary to replace the y_t/q estimates of N_t in eq. 2 with smoothed estimates $\hat{N}_t$ that remove at least some of the variance due to measurement errors in y_t. There are at least two options for obtaining such smoothed estimates.

First, we may simply replace y_t/q with three-point moving average (MA-3) estimates of the form

$$(3) \qquad N_t^{\text{MA-3}} = (y_{t-1} + y_t + y_{t+1})/(3q)$$

It is not recommended to use longer moving averages of the y_t data, since such averages will induce artificially smooth trends in the R_t estimates and hence mask any relatively rapid (regime shift) changes in R_t that may have occurred. For a simulated example with an abrupt R change halfway through the data series (Fig. 1*a*), even a MA-3 will give the appearance of a slower recruitment change than actually occurred. More complex variations on moving average smoothers, such as exponentially weighted procedures, are likely to result in even worse artificial trends in estimated R_t.

Second, since no particular nonlinear structure (stock–recruit) is assumed in eq. 1 for R_t, we may apply the theory of optimal state estimation for linear dynamical systems, i.e., Kalman filtering (Gelb 1974; Reed and Simons 1996) and Rauch–Tung–Striebel smoothing (Rauch et al. 1965), as suggested by Freeman and Kirkwood (1995). Equations needed for this approach are reviewed in Appendix A. This approach does require an assumption about how rapidly R_t is likely to have changed, in the form of assumed normality with known variance σ_R^2 for w_t in the nonstationary random walk model $R_t = R_{t-1} + w_t$ and also an assumed value of σ_v^2. But in simulated examples (like Fig. 1*a*), we have found that it typically gives somewhat better performance than simple three-point smoothing for a wide range of σ_R^2/σ_v^2 choices, with choices outside this range being obviously too smooth (for low σ_R^2) or approaching the Schaefer estimates (for high σ_R^2). For the example provided (Fig. 1*a*), using $SS = \Sigma(R_{true} - R_t)^2$ as a criterion for success in recovering the simulated R_{true} values and with a CV of 0.1 for y_t, we found the following averages over 100 simulation trials (with random v_t):

Method	SS
Schaefer	25.94
MA-3	2.20
Optimum linear	0.93

That is, for cases with abrupt recruitment change at some time in the past, we can expect convincing improvement in recruitment reconstruction performance by moving from the simple differencing method of eq. 2 to a moving average or optimum linear filtering method but not much improvement over the simple moving average approach by using more elaborate filtering methods. Note that the optimal filtering equations can be easily modified to account for temporal change in σ_v^2 (e.g., if y_t has constant CV, so that σ_v^2 varies as $\sigma_v = \text{CV}_y$, smaller σ_v^2 can be assumed for years of lower relative abundance) and can be used to estimate "point innovations" in R_t that cannot be represented by normally distributed errors w_t; using such tactics, the optimum filtering

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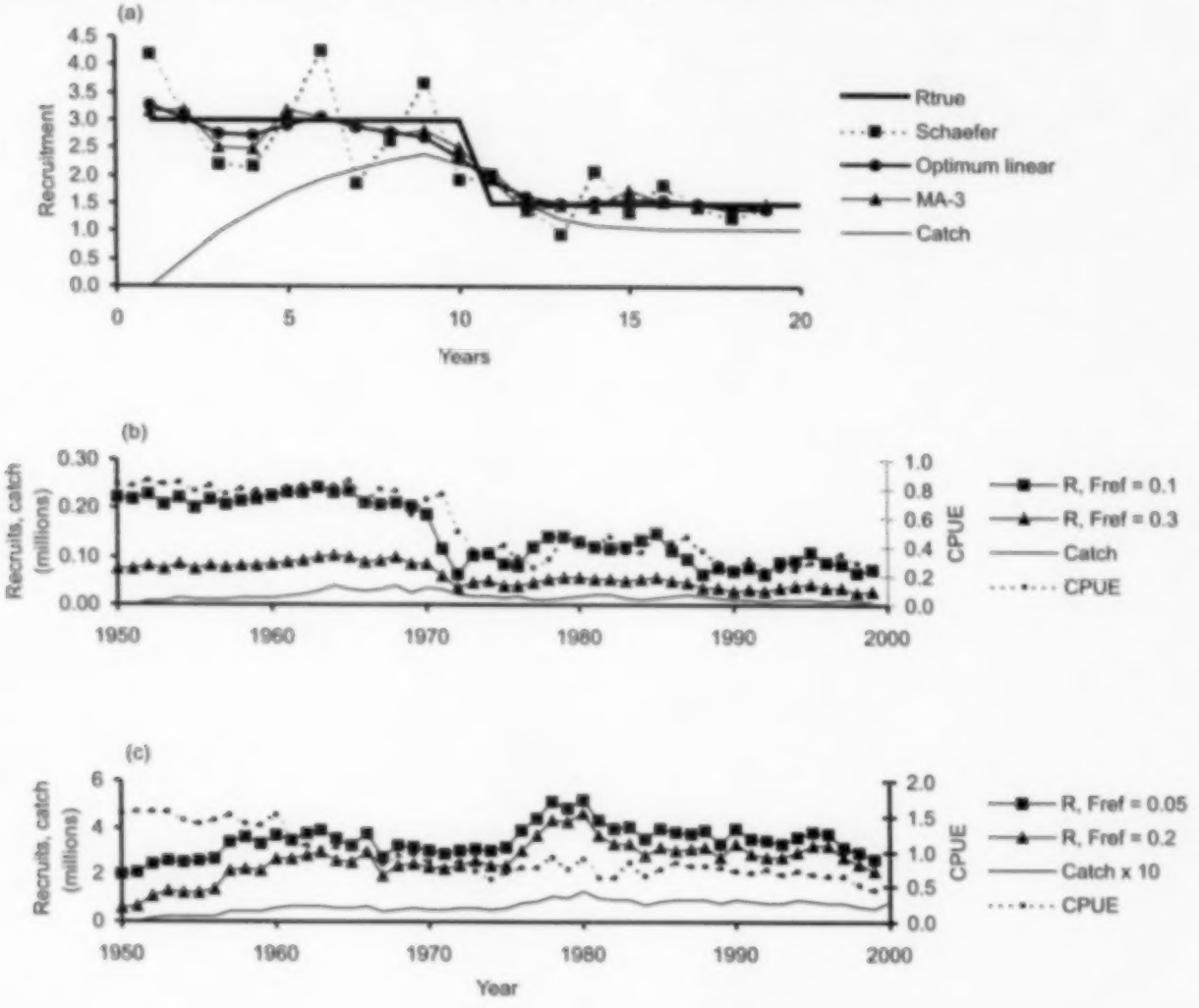
Fig. 1. Examples of recruitment reconstructions using the Schaefer approach. (*a*) A simulated population that shows two periods of stable recruitment with an abrupt transition between these periods (R_{true}, simulated true recruitment; Schaefer, unsmoothed estimates; optimum linear, Kalman filter smoothing; MA-3, three-point moving average smoothing). (*b*) Spatial mean catch per unit effort and calculated recruitments (using MA-3 smoothing) for striped marlin (*Tetrapturus audax*) in the Pacific Ocean for two hypotheses about recent longline fishing mortality rate F_{ref}. (*c*) Spatial mean catch per unit effort and calculated recruitments (MA-3 smoothing) for yellowfin tuna (*Thunnus albacares*) in the Pacific Ocean, again for two hypotheses about recent longline *F*.



approach can be improved somewhat compared with the example table above.

For those who would insist on using best possible statistical technology even for exploratory analysis, there remains the problem of making reasonable choices for the filtering parameters σ_k^2 and σ_γ^2. One option is simply to present a set of recruitment trend estimates that would result from alternative assumptions about these variances (or more importantly, about the ratio $\sigma_k^2/\sigma_\gamma^2$), as in Fig. 1*a*, without pretending that any member of this set is "best". Another is to construct a set of equally likely (in a Bayes prior sense) or plausible historical recruitment patterns using MA-3 results as a guide, simulate data gathering from these patterns, and then take the best estimates of the variances to be those that minimize an objective criterion like the SS defined above over a large number of simulation trials and plausible patterns. The important point is that there is no way to make a fully objective choice of the filtering parameters, free of any prior assumption about how rapidly recruitment may have changed in the past.

Notice in Fig. 1*a* that the Kalman filtering assumption of normally distributed changes in recruitment and also MA-3 smoothing lead to recruitment estimates where the "true" large recruitment drop in 1 year is estimated to have been a more gradual drop over 2–3 years. In such cases, outliers in the filter innovation (prediction error) sequence $y_t - \mathbf{Q}\mathbf{x}_{t|t-1}$ (see Appendix A) can be used to identify sudden changes, and the likelihood function calculated from this sequence

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(Reed and Simons 1996) can be used to estimate single point innovations for one or a few such years. However, such statistical refinements are not likely to be very helpful in the important detective work needed to identify possible causes for rapid change in apparent recruitment, including nonbiological effects such as changes in q.

In making a choice of smoothing method, we are forced to confront the trade-off between getting rid of y_t noise versus imposing artificial smoothness on the R_t estimates. This is a precision–accuracy trade-off problem exactly like we face in more complex assessments by including more nuisance parameters to explain variation in the data: we can make the estimates more precise by smoothing, but only at the expense of using estimators that are potentially less accurate (more biased). But in this case, there is no use pretending that we can resort to some objective criterion, like the Akaike information criterion, to make a best model choice.

For examination of concerns about recruitment overfishing, or for providing R_t time series for comparison (and fitting) with predictions from models like Ecosim that may ascribe large recruitment changes to trophic interactions, it is not really necessary to remove as much of the noise due to y_t variation as possible. It is important to be able to see large, relatively sudden changes in R_t of the sort that environmental regime shifts might have caused and that might be misinterpreted as stock–recruitment or trophic effects if miscalculated to have occurred smoothly over time.

Representing uncertainty in catchability q by alternative fishing mortality scenarios

For most cases where long-term relative abundance data are available, there is at least some recent reference period for which we can provide bounded estimates of the average fishing mortality rate F_{ref}, i.e., the average annual C/N for the period, through analysis of age–size data, tagging experiments, etc. For such periods, the average ratio $H_{ref} = C_{ref}/y_{ref}$ (where C_{ref} and y_{ref} are averages of C_t and y_t for the reference period) is an estimator of F_{ref}/q, since assuming $y = qN$ implies $C/y = C/(qN) = (C/N)/q = F/q$. Thus, any prior estimate F_{ref} leads immediately to a corresponding hypothesis about q, namely $q = F_{ref}/H_{ref}$.

Even absent any recent direct information on F, we can generally at least construct a reasonable range of q values by assuming a reasonable range of possibilities for F_{ref} based on the range of F that various fisheries with similar gear, swept area per unit effort, etc., have exhibited. We illustrate the widely divergent results that can result from admitting high uncertainty in F_{ref} (Figs. 1*b* and 1*c*). For these examples of Pacific Ocean pelagic species, we used the approach suggested in Walters (2003) to obtain spatial average catch rates from the 5 × 5 degree all-nation data available from the Secretariat of the Pacific Community (www.spc.org).

For the striped marlin (*Tetrapturus audax*) example (Fig. 1*b*), the recruitment reconstruction indicates a severe, relatively sudden decline in apparent recruitment to the stock available to longlines during the late 1960s. Essentially the same pattern is obtained for a wide range of F_{ref} possibilities. So in this example, the conclusion that there was an important recruitment change is very robust to uncertainty about q. Such a possibility has obvious implications for assessment of current stock status and reference points but is not mentioned in recent assessment documents like Hinton and Maunder (2003). So this is a good example of where exploratory recruitment analysis may expose apparent change that could escape attention when the usual assessment model fitting procedures are used. It might well turn out that the apparent change is an artifact caused by changes in q, catch reporting, or some other problem with the data; the key point is that with the results from the exploratory analysis, it should be relatively easy to examine the data more closely so as to evaluate such possibilities.

But for yellowfin tuna (*Thunnus albacares*) example (Fig. 1*c*), assuming high longline fishing mortality rates (>0.1) for recent (1990 and later) years causes the recruitment reconstruction equations to show a substantial increase during the early years of the fishery. Detailed age–size structured assessment models (Hampton 2002; Maunder and Watters 2002) for yellowfin also give relatively stable or slowly increasing recruitment after 1960 but do not recognize the possibility of a more substantial earlier increase that might signal a dome-shaped recruitment relationship (which would imply recruitment declines if the stock were managed more conservatively). So in this example, we obtain patterns with widely divergent policy implications depending on the F_{ref} (q) used for the reconstruction.

We illustrate how the recruitment estimates from eq. 2 or its smoothed analogs consist of two components, one from relative abundance ($y_t/q - Sy_{t-1}/q$ or $\hat{N}_t - S\hat{N}_{t-1}$) plus one from catch ($SC_t$) (Figs. 1*b* and 1*c*). In the striped marlin case (Fig. 1*b*), these components indicate similar recruitment trends. But in the yellowfin tuna case (Fig. 1*c*), weighting the catch term more heavily (high q or F_{ref}) leads to recruitment estimates that track the catch trend; the possibility that yellowfin tuna recruitment increased as the fishery developed could be rejected only if it could be demonstrated F_{ref} was in fact even lower than 0.05 or that early catch per unit effort was not in fact proportional to stock size. Another possibility in the yellowfin tuna case is that recruitment was in fact stable during the early years of the longline fishery, but catch per unit effort values showed hyperdepletion even within 5 × 5 degree spatial cells due to some factor such as selective removal of more vulnerable fish; such hyperdepletion would have led to less positive values of the $y_{t+1}/q - Sy_t/q$ term in the recruitment estimates for early t.

Discussion

While we introduced the method as a way to obtain estimates of long-term recruitment trends for situations where more complex assessment data are not available, it may in fact be more broadly applicable. Relative abundance data, at least for more recent years, are generally required for stock assessment methods, since more detailed composition data provide information about relative recruitment variation among adjacent cohorts but not about longer term trends. It should therefore not surprise anyone that we can bypass the complex age–size structured calculations to see what recruitment information is present in the relative abundance data using only information on survival (M, S) and catchability from other analyses. We are tempted to suggest that the simple "exploratory" methods should always be done, even when

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much more detailed information and assessment models are available, to expose whether the detailed information is in fact influencing the recruitment trend estimates at all. In cases where there does appear to be some strong influence, we should be just as suspicious of the composition data (or the model assumptions and multiple nuisance parameters used in fitting those data) as we know from much bad experience that we need to be in interpretation of relative abundance data.

It appears that relatively simple data smoothing and state estimation techniques can overcome one of the three major problems with using relative abundance data to directly back-calculate historical recruitments, namely variance caused by measurement error in relative abundance. We suggest a strategy for overcoming the second major problem, uncertainty about q, by providing a plausible range of recruitment reconstructions based on a plausible range of uncertainty about fishing mortality rate (F_{ref}). This leaves just one really large potential source of error, namely progressive or sudden but persistent changes in q over time. This is in fact a big problem for all assessment methods, not just simple exploratory back-calculations; one can mistune a 200-parameter model just as easily as a simple back-calculation, with all of those parameters varying so as to help explain any apparent inconsistencies.

Increases in q over time (fisher learning, better targeting, movement into areas of higher abundance, hyperstability) will obviously make recruitment appear to have increased or at least remained stable, and decreases in q (removal of more vulnerable fish, hyperdepletion) will make recruitment appear to have decreased. Regulatory and marketing changes can cause sudden, irreversible changes in q. The main tactics that we have to overcome these problems are to use well-designed survey data and (or) careful spatial analysis of fisher catch rates. Both of these tactics can fail badly, especially for stocks that have displayed large changes in spatial range and pattern. There is an emerging recognition of the need to complement them with methods for direct assessment of fishing mortality rates, e.g., by tagging (Martell and Walters 2002), but this will not help us reach back into historical times of informative stock change.

In cases where q has changed substantially over time in ways that may produce severe bias in any assessment model that uses the data, exploratory recruitment reconstructions can help to identify the potential bias. Being sensitive to changes in the relative abundance index, the recruitment calculation can "amplify" q changes into unrealistic or physically impossible recruitment estimates. In doing this, it may help to avoid rather than to promote misleading use of data.

What should we do when the exploratory analysis reveals widely divergent hypotheses about recruitment trends or differs greatly from estimates based on more detailed assessment models? One simple answer would be to simply ignore the exploratory results or to shrug them off as likely being due to violation of the constant q assumption; such tactics are in fact routine in the pathological assessment processes that are widespread today, where assessment scientists are compelled to deliver single best assessments (along with much incomprehensible and largely meaningless information about statistical limits and distributions when in fact the big uncertainties are structural rather than quantitative). A far wiser answer would be to embrace the alternative hypotheses as viable possibilities for explaining the historical data and to include any policy implications of these hypotheses (e.g., dome-shaped recruitment) in decision analyses aimed at identifying robust and adaptive management policies.

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Appendix A. Estimation of abundance and recruitment trends using the theory of optimal state estimation for linear dynamical systems.

To apply linear state estimation theory, we first write the state and recruitment dynamics equations in matrix form, where bold letters indicate vectors and matrices:

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(A1) $\mathbf{x}_{t+1} = \mathbf{A}(\mathbf{x}_t - \mathbf{c}_t) + \mathbf{w}_t$

Here, $\mathbf{x}_t = (N_t\ R_t)'$, $\mathbf{c}_t = (C_t\ 0)'$, and $\mathbf{w}_t = (0\ w_t)'$ and $\mathbf{A}$ is the state transition matrix:

$$\mathbf{A} = \begin{bmatrix} S1 \\ 01 \end{bmatrix}$$

We assume that $\mathbf{w}_t$ is normally distributed with covariance matrix

$$\mathbf{W} = \begin{bmatrix} 0\ 0 \\ 0\sigma_R^2 \end{bmatrix}$$

Next, we represent y_t as

(A2) $y_t = \mathbf{Q}\mathbf{x}_t + v_t$

where $\mathbf{Q} = (q\ 0)$ and v_t is assumed normally distributed with variance σ_y^2. Optimal linear state estimation then proceeds by first applying Kalman filter equations to provide optimal estimates $\mathbf{x}_{tt}$ of $\mathbf{x}_t$, where the notation *tt* denotes "at time *t* given data up to time *t*". Then, working backward in time starting from $\mathbf{x}_{TT}$ (the overall optimal estimate for time *T* given all years' data), the Rauch–Tung–Striebel smoothing algorithm gives optimal estimates $\mathbf{x}_{tT}$, i.e., of $\mathbf{x}$ at each time given all years' data. The filter and smoothing equations require calculation of two covariance matrices for $\mathbf{x}_t$: $\mathbf{P}_{tt-1}$ is the prediction covariance matrix for the prediction $\mathbf{x}_{tt-1}$ of $\mathbf{x}_t$ given data up to time $t - 1$ and $\mathbf{P}_{tt}$ is the updated covariance matrix for $\mathbf{x}_t$ given data to time *t*. The $\mathbf{x}_{tt-1}$ is calculated by applying eq. A1 with updated state estimates $\mathbf{x}_{t-1t-1}$. The covariance matrices $\mathbf{P}$ and state update from $\mathbf{x}_{tt-1}$ to $\mathbf{x}_{tt}$ are given by the Kalman filter recursive relationships

(A3) $\mathbf{P}_{tt-1} = \mathbf{A}\mathbf{P}_{t-1t-1}\mathbf{A}' + \mathbf{W}$

(A4) $\mathbf{K}_t = \mathbf{P}_{tt-1}\mathbf{Q}'/(\mathbf{Q}\mathbf{P}_{tt-1}\mathbf{Q}' + \sigma_y^2)$

(A5) $\mathbf{x}_{tt} = \mathbf{x}_{tt-1} + \mathbf{K}_t(y_t - \mathbf{Q}\mathbf{x}_{tt-1})$.

Here, $\mathbf{K}_t$ is the remarkable Kalman gain, which corrects the state estimates by applying a linear correction from the predicted state $\mathbf{x}_{tt-1}$ based on past data, proportional to the prediction error $y_t - \mathbf{Q}\mathbf{x}_{tt-1}$. These calculations can be initialized simply with $\mathbf{P}_{00} = \mathrm{diag}(10^{10})$ (high prior uncertainty) and $\mathbf{x}_{00} = (y_1/q\ \ y_1/q\ \ (1 - S)f$ (unfished equilibrium assuming $N_0 = y_1/q$).

Given the filtering results from eqs. A3–A5, the Rauch–Tung–Striebel algorithm then proceeds backward in time using the following backward recursion:

(A6) $\mathbf{x}_{tT} = \mathbf{x}_{tt} + \mathbf{P}_{tt}\mathbf{A}'\,\mathbf{P}_{t+1t}^{-1}\,(\mathbf{x}_{t+1T} - \mathbf{x}_{t+1t})$

starting from $\mathbf{x}_{TT}$. Notice that this remarkably simple calculation does not require "inverting" the model solution to predict $\mathbf{x}_t$ from $\mathbf{x}_{t+1}$. It is, however, complicated enough with the inverse matrix term to make spreadsheet implementation difficult.

An important point is that the state estimates (numbers and recruitment) resulting from eqs. A3–A6 are not just some smooth approximation to the optimum state estimates. Under normality assumptions for w and v, these estimates are in fact maximum likelihood estimates and cannot be improved upon by more complex estimation procedures.

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Covariation between grey seal (*Halichoerus grypus*) abundance and natural mortality of cod (*Gadus morhua*) in the southern Gulf of St. Lawrence

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Abstract: More than 10 years after the collapse of Atlantic cod (*Gadus morhua*) fisheries in Canada, the role of increased seal populations in the decline and lack of recovery of the stocks continues to be discussed. Using removals and abundance indices from synthetic populations, we found that sequential population analysis can uncover trends in natural mortality. We used this approach to examine variation in natural mortality (M) of southern Gulf of St. Lawrence cod. M increased from the late 1970s to the mid-1990s but declined slightly recently. Results were consistent with previous work indicating that M increased in the 1980s. Changes in estimated M for this cod stock matched fluctuations in grey seal (*Halichoerus grypus*) abundance. The increase in grey seal abundance from the late 1970s to the mid-1990s corresponded with the increase in estimated M of cod over this time period. The correspondence between seal abundance and M of cod supports the hypothesis that seal predation may be a cause of increased M. However, the diet information available suggests that seals consume mainly juvenile cod, whereas our evidence for an increase in M is for larger cod (ages 3 years and older).

Résumé : Plus de 10 ans après l'effondrement des pêches de morues franches (*Gadus morhua*) au Canada, on continue à discuter du rôle de l'augmentation des populations de phoques dans le déclin et l'absence de récupération des stocks. À l'aide de captures et d'indices d'abondance de populations synthétiques, nous avons découvert que l'analyse séquentielle de la population peut révéler les tendances de la mortalité naturelle. Nous avons utilisé cette méthodologie pour examiner la variation de la mortalité naturelle (M) chez les morues de la région sud du golfe du Saint-Laurent. M a augmenté de la fin des années 1970 jusqu'au milieu des années 1990 et a baissé un peu récemment. Ces résultats concordent avec des travaux antérieurs qui indiquent une augmentation de M durant les années 1980. Les changements dans la valeur estimée de M de ce stock de morues correspondent aux fluctuations de l'abondance des phoques gris. L'accroissement de l'abondance des phoques gris de la fin des années 1970 jusqu'au milieu des années 1990 correspond à une augmentation de la valeur estimée de M des morues durant la même période de temps. La correspondance entre l'abondance des phoques et la mortalité naturelle des morues appuie l'hypothèse selon laquelle la prédation par les phoques peut être la cause de la valeur accrue de M. Cependant, l'information disponible sur le régime alimentaire des phoques indique qu'ils consomment surtout des jeunes morues, alors que nos données indiquent qu'un accroissement de M se produit chez les morues plus grandes (âgées de 3 ans ou plus).

[Traduit par la Rédaction]

Introduction

Since the collapse of most of the Atlantic cod (*Gadus morhua*) fisheries off the east coast of Canada in the early 1990s, there has been considerable debate regarding the causes for their decline and subsequent lack of recovery. While it has been shown that increases in fishing mortality (F) played a major role in the collapse (Myers and Cadigan 1995; Sinclair and Murawski 1997; Shelton and Lilly 2000), some studies have also hypothesized that increases in the instantaneous rate of natural mortality (M) could have occurred during the same period (see review in Shelton and Lilly 2000).

Recently, Sinclair (2001) showed that M for cod in the southern Gulf of St. Lawrence was around 0.4 in the mid-1990s, about twice the levels previously assumed. His analysis suggested that M had started to increase in the early 1980s. This result has been incorporated in recent population reconstructions using sequential population analysis (SPA) by assuming that M doubled from 0.2 to 0.4 in 1986 (Chouinard et al. 2003).

Causes of this apparent increase in M are unknown. Possibilities include unreported catch, disease, toxins, starvation, parasitism, and predation. The possibility of unreported

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catch is unlikely. At the low spawning stock biomass occurring in the 1990s, about 20 000 t of unreported catch would be required to account for an apparent increase in M from 0.2 to 0.4 in the case of southern Gulf cod (G.A. Chouinard, unpublished data). It is unlikely that such a high level of unreported catch would have gone unnoticed in the absence of a directed cod fishery in the mid-1990s. There is no evidence for recent increases in disease, parasitism, or toxins. For northern Gulf cod, it has been suggested that cold environmental conditions in the early to mid-1990s may have resulted in increased M of cod as a result of poor growth and physiological condition (Lambert and Dutil 1997). Although there is some evidence for an effect of water temperature on growth of southern Gulf cod (Campana et al. 1995; Sinclair et al. 2002; Swain et al. 2003), growth and physiological condition in this stock declined in the late 1970s and early 1980s, well in advance of the onset of cooling in the late 1980s.

Harp (*Phoca groenlandica*) and grey seals (*Halichoerus grypus*) are predators of cod in the southern Gulf of St. Lawrence. Harp seals occur in the southern Gulf during late winter and early spring, a time when adult cod are overwintering outside the Gulf in the Sydney Bight area off the east coast of Cape Breton Island. Grey seals, on the other hand, are present year-round in southern Gulf waters and on the Scotian Shelf (Stobo et al. 1990; Lavigueur and Hammill 1993; Goulet et al. 2001), where they can co-occur with overwintering southern Gulf cod. Seals in Atlantic Canada have a varied diet and cod usually represent less than 20% of their diet (Hammill and Stenson 2000). Data on the diet composition of harp seals in the southern Gulf are limited, but the existing information suggests that consumption of cod from this stock is low (Beck et al. 1993; Hammill and Stenson 2002). However, cod can be an important item in the diet of grey seals in the southern Gulf of St. Lawrence (Hammill and Stenson 2002).

In other areas, detailed diet composition data have allowed removals of cod by seals to be incorporated into population assessment models (e.g., Mohn and Bowen 1996; Fu et al. 2001). Unfortunately, while there are estimates of consumption of cod by seals, the long time series of cod removals-at-age by seals required for these analyses do not exist for the southern Gulf of St. Lawrence. While these data are not available, there are relatively good estimates of the abundance of grey seals in Atlantic Canada (Mohn and Bowen 1996; Hammill and Stenson 2002; Bowen et al. 2003). Pup production provides indices of abundance for grey seal populations. Pup production on Sable Island has been increasing exponentially at an annual rate of 12.8%, from about 2000 animals in 1977 to 25 400 in 1997 (Bowen et al. 2003). A second large component of the grey seal population whelps primarily on the pack ice in the southern Gulf of St. Lawrence. Pup production in this area increased from 7200 in 1984 to 11 100 in 1996 and then declined rapidly to 7300 in 1997 and 5400 in 2000 (Hammill et al. 2003; Hammill and Stenson 2002). The recent decline in the southern Gulf pup estimates is likely due to increased pup mortality, a shift in pupping of females to areas outside the Gulf, or a combination of the two owing to poor ice conditions in recent years (M.O. Hammill, unpublished data).

In this paper, we explore the possibility that the increase in M of southern Gulf cod is a result of increased predation by grey seals. We obtain estimates of M in the southern Gulf stock for various time periods between the mid-1970s and 2001 and show that the trend in M corresponds closely to trends in the abundance of grey seals in the southern Gulf, consistent with the hypothesis that the increase in M of cod in the southern Gulf is a result of increased predation by grey seals.

Methods

Estimating M from SPA

Conventional age-structured assessment models typically assume that M is known (see review in Megrey 1989). Because studies to estimate M are rarely carried out, the value used for M in these models is usually an assumption rather than an empirical estimate. The principal reason for making an assumption about M rather than attempting to estimate it is that it can be confounded with other parameters of interest such as F, population size, and catchability coefficients. However, Schnute and Richards (1995) showed, using simulation-estimation techniques, that it is feasible to estimate M in catch-age models. Similarly, Fu and Quinn (2000) found that interannual variation in M could be estimated in a stock assessment model of *Pandalus borealis* using the AD model builder (ADMB) methodology.

In this study, we estimated trends in M as part of the "calibration" of SPA. SPA produces estimates of population abundance by adding up fish catches along cohorts or year classes while adjusting for M. The basic population equation used in the SPA is as follows:

$$(1) \qquad N_{a,t} = N_{a+1,t+1}\, e^{M_{a,t}} + C_{a,t}\, e^{M_{a,t}/2}$$

where $N_{a+1,t+1}$ is the number of survivors at age $a + 1$ in year $t + 1$, $C_{a,t}$ is the catch in numbers at age a in year t, and M is the instantaneous rate of natural mortality. The catch is assumed to occur in midyear and measured without error, the usual assumption in many cod assessments. Starting estimates of the survivors in the last year of analysis are needed to begin SPA. Parameter estimates are obtained by calibrating the SPA using population abundance indices (e.g., mean catch rates in a research survey). Calibration involves finding the parameter estimates that produce the best match between the SPA estimates of population size and the population abundance indices. We used the ADAPT framework (Gavaris 1988) to calibrate SPA. The ADAPT software allows for the estimation of M in addition to providing estimates of the survivors at age in the terminal year and catchability coefficients (q) at age for the population abundance indices. These parameters are estimated using nonlinear least squares by minimizing the following objective function:

$$(2) \qquad \sum_{a,t,i} [\ln(I_{a,t,i}) - \ln(q_{a,i} N_{a,t})]^2$$

where $I_{a,t,i}$ is the abundance index at age a in year t for abundance index i, $q_{a,i}$ is the catchability coefficient at age a for index i, and $N_{a,t}$ is the number of survivors at age a at the beginning of year t.

We first conducted a simulation study to investigate the feasibility of estimating trends in M using the ADAPT soft-

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ware. To limit the number of parameters and to reduce the possibility of spurious results owing to "year effects" (i.e., interannual fluctuations in catchability), we investigated the reliability of estimating M for blocks of years (periods varying from 5 to 9 years) rather than individual years.

We generated three synthetic populations with known underlying patterns of M. These synthetic populations, each comprising 13 age-classes, were generated by defining an initial (1st year) population and then making a forward projection over 31 years using randomly selected F (0.2 ± 50%), randomly selected recruitment (20 000 ± 10% or 20%), a constant exploitation pattern by age, and the following three M profiles: M = 0.2 from year 1 to 14 and then 0.4 for years 15–31, M = 0.2 from year 1 to 3 and then increasing in gradual increments of 0.02 to reach 0.4 in year 14 and remaining at 0.4 thereafter, and M = 0.2 for years 1–10, M = 0.4 for years 11–23, and M = 0.2 for years 24–31.

We used two different catchability profiles (constant for all years) to construct two "exact" survey indices. These abundance indices were then randomly perturbed (30% coefficient of variation for each index). We used ADAPT to estimate the population parameters with the catch-at-age calculated in generating the population and the perturbed indices. Standard errors of the parameter estimates are obtained using the covariance matrix (Gavaris 1988). In addition to the usual parameters (terminal population estimates and catchability coefficients for the abundance indices), we used ADAPT to estimate M for equal periods of 5–9 years (one value for each 5- to 9-year period). The periods were defined starting with the terminal year (e.g., for 5-year periods: years 27–31, 22–26, 17–21, 12–16, and 7–11). A value for M was assumed rather than estimated for the 3- to 6-year period at the beginning of the time series ($M_{initial}$). The sensitivity of the estimated trend in M to the value assumed at the beginning of the time series was examined by conducting analyses assuming $M_{initial}$ = 0.1, 0.2, and 0.4. Using the different assumptions of $M_{initial}$ resulted in a scaling up or down of the estimated M; however, the trends in M were similar. Because the trends in estimated M were not sensitive to this assumption, we used the analyses assuming $M_{initial}$ = 0.2, as this was the value for M assumed in earlier cod assessments based on studies conducted prior to 1980 (see Sinclair 2001). Besides the estimation of M by time periods, the rest of the calibration model was similar to that used in the assessment of southern Gulf of St. Lawrence cod (Chouinard et al. 2003).

For each of the three populations generated using the different M profiles, the calibration of the population model using ADAPT was repeated 500 times. At each iteration, the exact abundance indices were randomly perturbed and the ADAPT calibration repeated. Finally, we compared the trends in the estimates of M with those originally used to generate the synthetic populations.

Natural mortality for southern Gulf of St. Lawrence cod and grey seal population estimates

As in the simulation study, we estimated M for the southern Gulf of St. Lawrence cod stock in blocks of 5–9 years. Data for the period 1971–2002 were available. The formulation of the population model was similar to that used in the most recent assessment of this stock (Chouinard et al. 2003). In summary, eight population abundance indices were used in the SPA calibration: a research vessel abundance index (a = 2–10, t = 1971–2002), an otter trawl catch rate series (a = 5–12, t = 1982–1993), and six sentinel survey indices using various gear types and mesh sizes (various ranges for a, t = 1995–2002 or 1996–2002). Sentinel surveys are stock abundance monitoring activities conducted by the fishing industry. All observations were given equal weight. The relationship between abundance indices and population numbers was assumed to be proportional except for the otter trawl catch per unit of effort index where a trend in catchability was estimated:

$$(3) \qquad I_{a,t} = (k_a \tau_a^{T-T_1}) P_{a,t}$$

where I is the catch rate index at age a in year t, k is the catchability coefficient at age a, τ is the trend coefficient, T is time, and P is the population abundance at age a and time t.

Fishing mortality on the oldest age was set to the average for the two previous age groups. As in the simulation study, calibration was achieved by minimizing the sum of squares of the objective function given by eq. 2.

In addition to estimating M for periods of 5–9 years, $q_{a,t}$ and the trend coefficients by age for the catch per unit of effort index (τ_a), $N_{3,2003}$,...$N_{14,2003}$ and $N_{15,2000}$,...$N_{15,2003}$, were estimated. Given the results obtained in the simulation study (see previous section), M was assumed to be 0.2 in the 2- to 8-year time period at the beginning of the time series ($M_{initial}$). We compared the fit and the population estimates of the various population models described above with the model used in the 2003 assessment where M is set equal to 0.2 for the period 1971–1985 and 0.4 for 1986–2002 (Chouinard et al. 2003). For completeness, we also compared the results of the variable-M models with the results obtained using constant M. Historically, assessment of this cod stock used a constant M of 0.2 (Sinclair 2001). In our analyses, we investigated models with constant M of 0.1, 0.2, 0.3, 0.4, and 0.5. In total, between 85 and 91 parameters were estimated in the models and there were 732 observations. We compared the fit of the various models using the corrected Akaike information criterion (AIC_c) calculated as described in Burnham and Anderson (1998). The differences between the AIC_c value for each model and that for the model with the lowest AIC_c (ΔAIC_c) provide a means of ranking model fits. If a particular model has $4 < \Delta AIC_c < 7$, there is evidence that the model is not the best and a value of $\Delta AIC_c > 10$ is considered very strong evidence that the model is not the best (Burnham and Anderson 1998).

Grey seal population estimates for the Gulf of St. Lawrence were obtained using the model described in Mohn and Bowen (1996) but with reproductive rates from data collected in the Gulf of St. Lawrence in the 1980s and 1990s (Hammill and Gosselin 1995). The model was initially fit to data available to Mohn and Bowen (1996) to verify model consistency. It was then fit in two steps to a larger data set that included more recent estimates of grey seal pup production in the Gulf of St. Lawrence. The first step included fitting the model to the increasing series of pup production estimates from 1984 to 1996 by adjusting initial population

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size and adult and pup mortality. The model was then fit to the pup production estimates from 1997 and 2000 by adjusting pup mortality for 1997 and onwards.

Finally, we compared the estimated trend in grey seal abundance in the Gulf with the trend in M estimated by SPA. For each model, we computed Spearman's coefficient of rank correlation between the M estimates and the average seal abundance during the corresponding period to provide an indication of association.

Results

Estimating M from SPA

The simulations indicated that trends in M could be reliably estimated as part of the SPA calibration (Fig. 1). The estimation uncovered the general trends in underlying M for the three simulated populations for all time period lengths. Where abrupt changes in M were used in generating the populations (e.g., a doubling or halving of M in one year) (Figs. 1*a* and 1*c*), estimates of M obtained from the 500 replicates were generally close to the true value. However, where a gradual increase had been imposed (Fig. 1*b*), although the general trend was revealed, the estimates of M in the period of increase were lower than the average true M during the period of increase.

Natural mortality for southern Gulf of St. Lawrence cod and grey seal population estimates

All of the population models with parameter estimates for M converged. The mean squared error (MSE) provides an estimate of the goodness of fit for these models. Overall, our analyses resulted in a slight reduction (1%–7%) in MSE (Table 1) over the model used in the assessment of the stock, which assumed a sudden increase in M (Chouinard et al. 2003). However, there was a greater than 15% reduction in MSE relative to models that assumed a constant M, the usual assumption in earlier stock assessment of this resource. The ΔAIC_c statistics, which take into account the difference in the number of parameters estimated, indicated that the model where M was estimated in periods of 7 years provided the best fit (Table 1). All of the models where M was estimated gave lower ΔAIC_c than the assessment model where a sudden change in M is assumed. The ΔAIC_c for the models assuming constant M were large relative to that of the other models and their results are not considered further.

Some high correlations (r = –0.6 to –0.8) occurred between estimates of M and q, mainly for the research vessel index, which covers the entire time series. Although M and q were closely correlated, the analyses consistently showed similar time trends in M despite using different time periods. In addition, the patterns of q by age for the various abundance indices, including the research vessel series, were similar between analyses estimating M for various time periods and between these analyses and the model used in the assessment (Fig. 2). As expected, different values of estimated M resulted in a scaling up or down of the catchability coefficients (higher estimates for M result in higher estimates of population size and thus lower estimates of catchability). Time trends in population abundance and F were also generally similar between our analyses and the standard assessment model (Fig. 3), although population abundance estimates were higher in the 1975–1990 period in most of the analyses where M was estimated. Fishing mortality estimates (ages 7+, weighted) during the period 1989–1992 were more than double the levels in the previous 4 years (1985–1988: F = 0.26 to 0.36; 1989–1992: F = 0.64 to 0.76). This is similar to the trends estimated in the stock assessment where M is not estimated. Estimated M increased progressively from values in the range of 0.25 to 0.30 in the late 1970s to approximately double that (0.45–0.56) in the late 1980s and early 1990s followed by a small decrease to values in the range of 0.34–0.39 in the late 1990s (Fig. 4).

Estimates of grey seal abundance from the Gulf of St. Lawrence showed a continuous increase from the early 1970s to the late 1990s (Fig. 4). The population is estimated to have declined slightly from 1997 to 2000 in response to the decline in pup production in the southern Gulf. Changes in estimated M matched estimated variation in the abundance of grey seals. The general increase in grey seal abundance from the late 1970s to the mid-1990s corresponded to the gradual increase in estimated M over this time period. Similarly, the recent decline of the component of the grey seal population that reproduces in the Gulf of St. Lawrence was matched by a decline in estimated M (Fig. 4). Spearman rank correlation indicated a significant (α = 0.05) positive association between estimated M and average seal abundance for the periods for models using 5- and 6-year periods (Fig. 4). The usefulness of this statistic was limited for the other models because there were only three and four observations. Significance would have required complete correspondence of the ranks.

Discussion

Using simulation-estimation studies, Schnute and Richards (1995) and Fu and Quinn (2000) showed that variation in M could be estimated in population models. In our analyses, the simulation exercise also suggested that trends in M can be recovered through the calibration of SPA using the ADAPT framework. Similar to most population models, our analysis assumed that q at age is constant over time. This is a reasonable assumption for research and sentinel surveys because survey protocols and gear are standardized.

Schnute and Richards (1995) found that estimation models performed better when there was a reasonably reliable abundance index such as those produced from well-planned research surveys. For southern Gulf of St. Lawrence cod, the research survey is the main index of abundance and has been conducted continuously for 32 years following a stratified random design using standard methods (Hurlbut and Clay 1990). The survey area extends to shallow waters and contains little untrawlable area. The coefficients of variation of the research survey abundance indices are low, ranging from 10% to 25% for the most common age groups (Chouinard et al. 2003). Similarly, several other abundance indices derived from sentinel surveys that have been conducted since the mid-1990s have followed standardized methods. In our synthetic population analyses, the exact abundance indices were perturbed using a coefficient of variation of 30% so that input data would be more variable than those used in the assessment of cod in the southern Gulf of St. Lawrence. This should provide a conservative evaluation of the technique.

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Fig. 1. Estimates of M from the simulation experiment for the three synthetic populations (a, b, and c) based on 500 bootstrap runs of the ADAPT framework. Box plots show the distribution of estimates of M for each time period; the line within the box marks the median, the lower and upper boundaries of the box show the 25th and 75th percentile, respectively, error bars below and above the box indicate the 10th and 90th percentiles, and circles show the extreme values. The line indicates the value of M used to generate the synthetic populations.

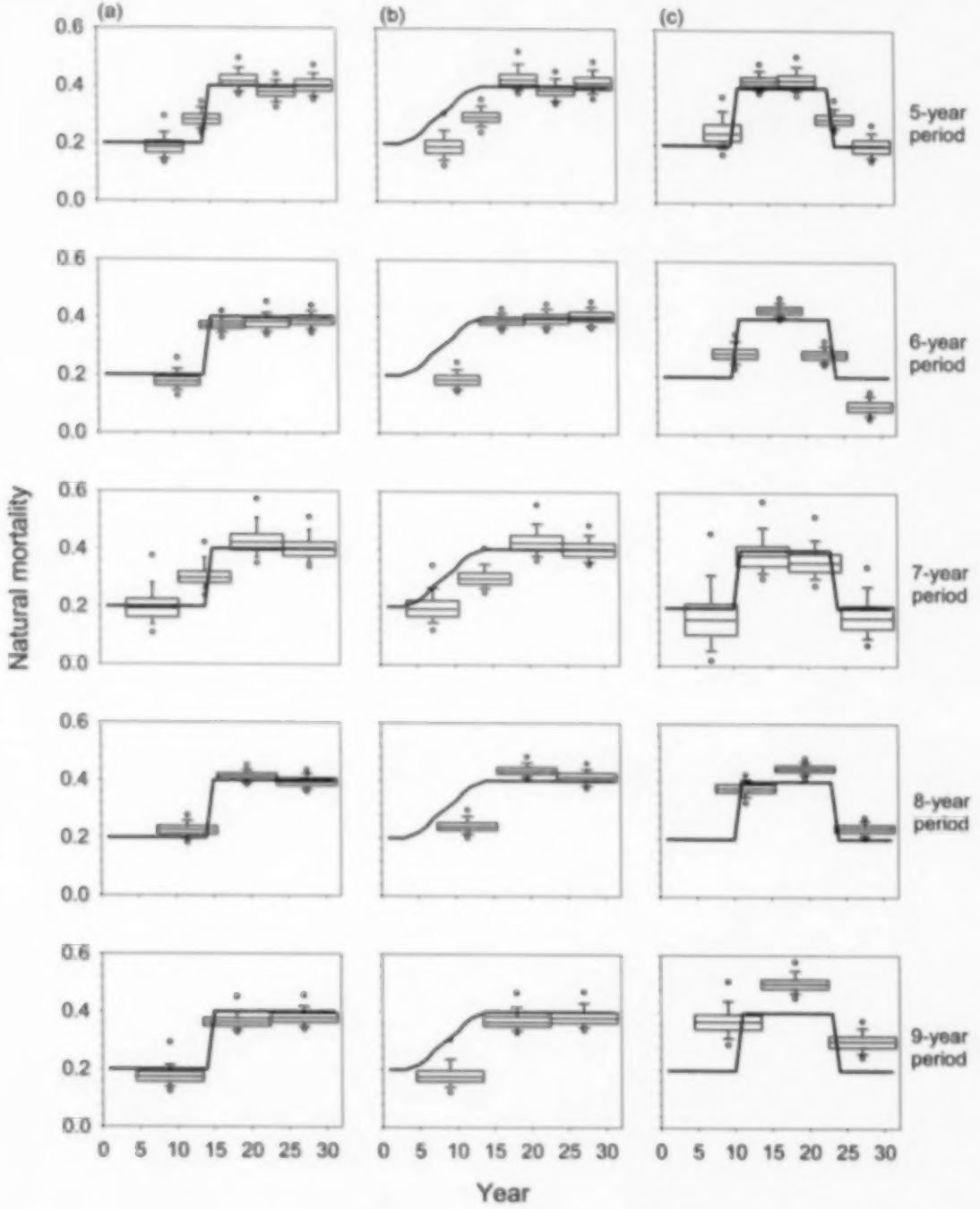

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Table 1. Number of parameters (P), number of estimates of M, MSE, and ΔAIC_c for the population models for southern Gulf of St. Lawrence Atlantic cod (*Gadus morhua*).

Model	P	M estimates	MSE	ΔAIC_c
Estimates of M in 5-year periods	91	6	0.182	27.47
Estimates of M in 6-year periods	90	5	0.183	30.01
Estimates of M in 7-year periods	89	4	0.176	0.00
Estimates of M in 8-year periods	88	3	0.181	19.05
Estimates of M in 9-year periods	88	3	0.188	46.83
Assessment (M = 0.2 from 1971 to 1985, M = 0.4 from 1986 to 2002 (Chouinard et al. 2003))	85	0	0.190	50.25
Constant M = 0.1	85	0	0.230	190.10
Constant M = 0.2	85	0	0.216	144.13
Constant M = 0.3	85	0	0.210	123.51
Constant M = 0.4	85	0	0.213	133.89
Constant M = 0.5	85	0	0.227	180.49

These analyses also assumed that catch-at-age was measured without error, as is the case in this and most other cod assessments.

Estimates of M for southern Gulf of St. Lawrence cod followed a similar pattern regardless of the block size (5–9 years) used to estimate M: M increased from the late 1970s to the early 1990s and then declined in the late 1990s. The consistency of these results suggests that the patterns uncovered are not spurious (Fu et al. 2001). In addition, the fits of all of the models using a constant M were significantly poorer than those where an increase in M was either assumed or estimated.

The first estimate of M in our study (6-year time period from 1973 to 1978) was considerably lower than that obtained using other time blocks in the same period. The reasons for this difference are unclear but the larger confidence intervals indicated that it was a less precise estimate. Sinclair (2001) noted that survey estimates of total mortality (Z) produced a negative estimate for 1977, which is contained in this period.

Our estimates of M in the mid-1990s for southern Gulf of St. Lawrence cod correspond well to Sinclair's (2001) estimate of M of about 0.4 in that period, using a modified catch curve analysis of the survey data. As well, the estimates of M from the SPA showed a trend similar to that of the residuals from a regression of Z on an index of fishing intensity (Sinclair 2001). To incorporate the increase in M, recent assessments of the stock (e.g., Chouinard et al. 2003) assumed a sudden doubling of M from 0.2 to 0.4 in 1986. This is ecologically unlikely and a gradual increase in M, as suggested by our analyses, would be more realistic.

Since the first closure of the fishery in the early 1990s, high M of adult cod has been one of the factors impeding the recovery of the southern Gulf cod stock from its collapse. The close correspondence between the estimated trends in the abundance of the Gulf of St. Lawrence component of the grey seal population and estimated M of cod supports the hypothesis that grey seal predation may be a cause of the increased M of cod in the southern Gulf of St. Lawrence. A difficulty with this hypothesis is that diet analyses suggest that grey seals feed primarily on small cod (Hammill and Stenson 2002), whereas evidence presented here and elsewhere (Sinclair 2001) indicates an increase in M of adult cod.

As in most other studies of pinniped diets (e.g., Murie and Lavigne 1985; Croxall 1993; Bowen 2000), estimated diet composition of seals in the Gulf of St. Lawrence is based on the hard body parts found in the digestive tracts and faecal material of seals. For example, the conclusion that the cod eaten by seals are mainly juveniles is based on the size distribution of cod otoliths recovered from seal stomachs (Hammill and Stenson 2002). This approach assumes that the hard parts recovered from seal stomachs are representative of the species composition and size distribution of prey consumed. However, selective rejection of fish heads has been suggested to occur in a number of seal species (Pitcher 1980; Lunneryd 2001; Moore 2003), and fishermen often report observations of seals feeding on large cod, consuming only the belly (including the energy-rich liver). This "belly-biting" has been documented for harp seals off Newfoundland (Fu et al. 2001). On the other hand, there is evidence from faecal samples of grey seals taking large cod (>40–90 cm; Bowen et al. 1993; Hammond et al. 1994*a*, 1994*b*), indicating that seals do not always consume only the soft parts of large fish. A link between grey seal abundance and the M of adult cod would require that grey seals frequently do not consume the head of large cod (>35 cm). Studies to determine the frequency of this behaviour should be encouraged. Alternative methods, such as fatty acid analyses (Iverson et al. 2004), may also lead to improved estimates of the diet composition of seals, free of the possible biases in diet reconstructions based on the hard parts found in digestive tracts and faeces.

Most estimates of consumption of southern Gulf cod by seals (Hammill and Stenson 2002) are lower than the amount required to account for the estimated increase in M. Estimated consumption by seals depends on their estimated population abundance, distribution, energy requirements, and diet composition. Distribution, energy requirements, and diet composition of seals can vary considerably both geographically and seasonally (Shelton et al. 1997). Diet samples are often collected in coastal areas, and it is assumed that diets in offshore areas are similar, which may not always be the case (Stenson et al. 1997). Most grey seal diet

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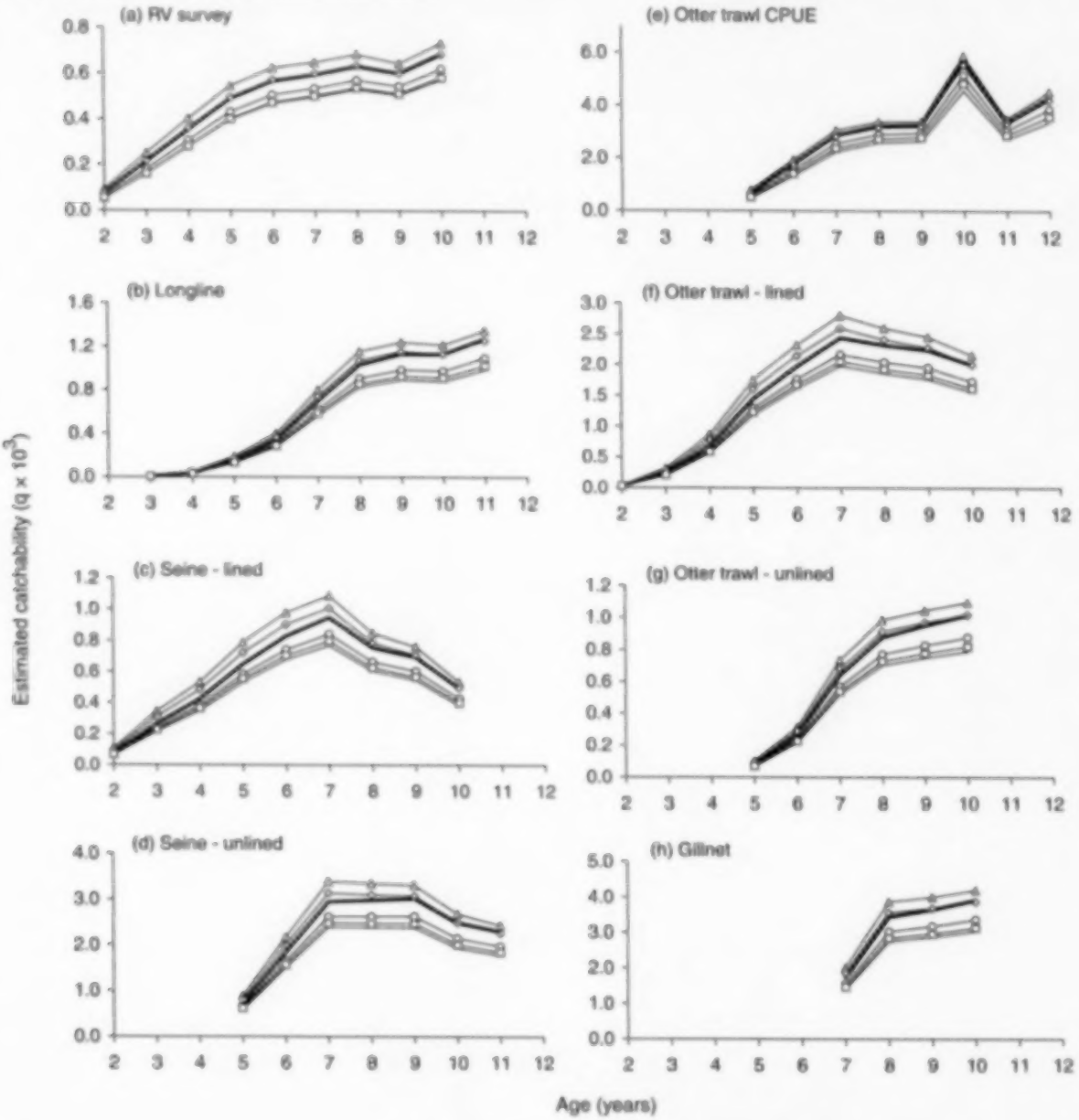
Fig. 2. Estimated catchability (q) at age for the various abundance indices obtained from the population models for southern Gulf of St. Lawrence Atlantic cod (*Gadus morhua*) where M was estimated for periods of 5–9 years (circles, 5 years; triangles, 6 years; diamonds, 7 years; squares, 8 years; thin line, 9 years) and from the most recent assessment (thick line) of the stock (Chouinard et al. 2003). RV, research vessel; CPUE, catch per unit effort.



samples for the southern Gulf of St. Lawrence are summer samples from inshore areas (e.g., the Northumberland Strait) where cod are rare (Hammill and Stenson 2002) and diet information is lacking in the main areas of cod distribution (Bowen et al. 1993). Thus, consumption of cod by seals may currently be underestimated owing to seasonal and spatial biases in diet sampling and possible "belly-biting".

Previous analyses of the impact of seal predation on cod in other areas have estimated the removals-at-age of cod by seals based on diet analyses and incorporated these removals in population models (e.g., Mohn and Bowen 1996; Fu et al. 2001). However, for several stocks, attempts to incorporate estimated seal consumption of cod in population models have resulted in inconsistent results. For example, Shelton

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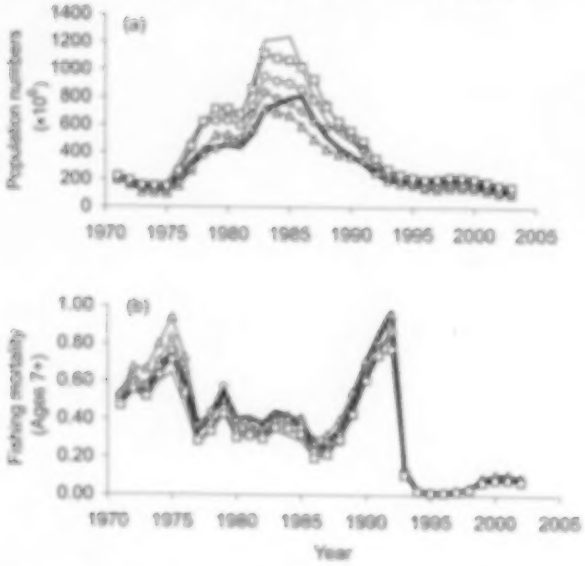
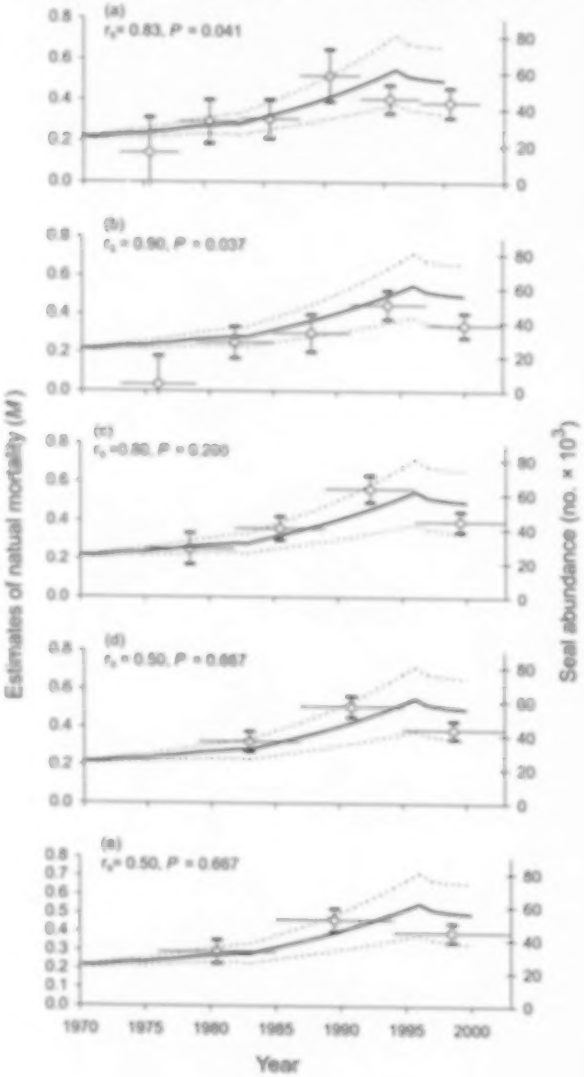
Fig. 3. (*a*) Population abundance and (*b*) *F* trends obtained from the population models for southern Gulf of St. Lawrence Atlantic cod (*Gadus morhua*) where *M* was estimated for periods of 5–9 years (circles, 5 years; triangles, 6 years; diamonds, 7 years; squares, 8 years; thin line, 9 years) and from the most recent assessment (thick line) of the stock (Chouinard et al. 2003).



and Lilly (2000) reported that estimates of cod consumed by seals were lower than the estimated "missing" fish for northern cod of ages 4 and 5. They also suggested that the discrepancy could be caused by seals feeding only on soft parts when consuming older cod.

We have focused on the congruent trends between *M* of adult cod and abundance of the Gulf grey seal herd. Grey seals from the Sable Island herd also occur in the Sydney Bight area where they may feed on southern Gulf cod in winter. In addition, while tag recoveries of Sable Island seals indicate that most of these animals remain on the Scotian Shelf throughout the year, some animals have a tendency to move into the Gulf. Most of these animals migrate to the northern Gulf near Anticosti Island or into the St. Lawrence Estuary during late spring and exit the Gulf in fall (Stobo et al. 1990). Abundance trends were parallel between the two herds until the mid-1990s. Since then, pup production has continued to increase on Sable Island, whereas it has declined by over 50% in the Gulf (Hammill and Stenson 2002; Bowen et al. 2003). The decrease in pup production is estimated to have caused a decline in the population in the Gulf (Hammill and Stenson 2002; this analysis). Our analysis suggests that the decline of the grey seal population in the Gulf between 1996 and 2000 was smaller than that estimated by Hammill and Stenson (2002). However, there is a similar correspondence to variation in estimated *M* irrespective of the series used. Finally, harp seals could also be important predators of cod in the southern Gulf. Although estimates of the consumption of cod by harp seals are low compared with consumption by grey seals if based on the limited diet data available for the southern Gulf, greater estimates are obtained assuming the high-cod diets seen at some times in some other areas (Hammill and Stenson 2002). Abundance trends were also roughly parallel between the harp and grey seal herds in the Gulf until the mid-1990s when harp seal abundance stabilized but grey seal abundance appeared to decrease (Hammill and Stenson 2002). Thus, the conclusion that seal predation on southern Gulf cod likely increased from the late 1970s to the mid-1990s remains valid consid-

Fig. 4. Estimated *M* for periods of (*a*) 5, (*b*) 6, (*c*) 7, (*d*) 8, and (*e*) 9 years (circles with horizontal lines spanning the period covered) from the population analyses of southern Gulf of St. Lawrence Atlantic cod (*Gadus morhua*) and trends in the grey seal (*Halichoerus grypus*) population (solid lines) with confidence interval (broken lines). Error bars for estimates of *M* and confidence intervals for grey seal population estimates are ±2 SE and r_s is Spearman's correlation coefficient calculated using the estimated *M* and the average seal abundance during each period.



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ering potential predation by harp seals and Sable Island grey seals in addition to predation by the Gulf grey seal herd.

It is becoming increasingly recognized that *M* increased for many cod stocks in Atlantic Canada during the 1980s. Besides the estimates from Sinclair (2001) and this analysis, Fu et al. (2001) concluded that *M* might have been high recently for cod on the eastern Scotian Shelf. Shelton and Lilly (2000) noted that an increase in *M* in the late 1980s is now routinely incorporated in several stock assessments. We note that pinniped population abundance is also high in the areas occupied by several western Atlantic cod stocks where increases in *M* are assumed.

Our results are consistent with previous analyses (e.g., Sinclair and Murawski 1997) that suggested that an increase in *F* was the main reason for the decline of cod in the southern Gulf. However, our analyses also suggest that an increase in *M* played a role in the collapse of this stock and in its failure to recover. While diet data are limited and feeding studies suggest that grey seals mostly target small cod, observations of belly feeding by seals and the results of this study showing the parallel trends between grey seal abundance and estimated *M* for this cod population suggest that predation by seals on adult cod may be more important than previously thought.

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Distribution–abundance relationships for North Sea Atlantic cod (*Gadus morhua*): observation versus theory

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Abstract: Density-dependent habitat selection has implications for fisheries management and for the recovery of depleted fish populations. According to ideal free distribution theory, populations contract into areas of highest habitat suitability as their abundance decreases. This can increase their vulnerability to fishing and predation. We detected density-dependent habitat selection by juvenile Atlantic cod (*Gadus morhua*) (ages 1 and 2) in the North Sea and compared the observed distribution–abundance relationships with those predicted from a model based on ideal free distribution theory and knowledge of optimal temperatures for growth, where temperature was used as a measure of suitability. As predicted by the model, in years when stock size was low, the catches were largely confined to regions with near-optimal bottom temperatures. Conversely, when population size was high, catches were spread across a larger area including regions with suboptimal temperatures. The spatial extent of optimal habitat appears to have decreased from 1977 to 2002, reflecting a gradual warming of the North Sea. The combined negative effects of increased temperature on recruitment rates and the reduced availability of optimal habitat may have increased the vulnerability of the cod population to fishing mortality.

Résumé : La sélection de l'habitat en fonction de la densité a des conséquences sur l'aménagement des pêches et la récupération des populations de poissons décimées. Selon la théorie de la distribution idéale libre, les populations se concentrent dans des zones d'habitats les plus adéquats à mesure que leur taille diminue, ce qui peut augmenter leur vulnérabilité à la pêche et à la prédation. Nous avons détecté une sélection de l'habitat en fonction de la densité chez de jeunes morues (*Gadus morhua*) (âges 1 et 2) de la mer du Nord et nous avons comparé les relations répartition–abondance observées à celles prédites à partir d'un modèle basé sur la théorie de la distribution idéale libre et les connaissances des températures optimales pour la croissance, dans lequel la température sert de mesure d'adéquation. Comme le prédit le modèle, les années où la taille du stock est basse, les captures sont en grande partie restreintes aux régions où les températures du fond sont presque optimales. Inversement, lorsque la taille de la population est grande, les captures sont étalées sur une surface plus étendue et même dans les régions avec des températures sub-optimales. L'étendue spatiale de l'habitat optimal semble avoir diminué de 1977 à 2002, ce qui indique un réchauffement graduel de la mer du Nord. Les effets négatifs combinés de la température plus élevée sur les taux de recrutement et de la disponibilité réduite de l'habitat optimal peuvent avoir augmenté la vulnérabilité de la population de morues à la mortalité due à la pêche.

[Traduit par la Rédaction]

Introduction

The relationship between the abundance and geographical distribution of animals is a widespread ecological phenomenon (Sutherland 1996) observed in many marine fish populations (MacCall 1990; Swain and Sinclair 1994; Marshall and Frank 1995). Attempts to explain distribution–abundance relationships have primarily focused on density-dependent habitat selection (DDHS), with populations expanding into suboptimal habitats at high abundance and contracting into optimal habitats when population size is low, owing to intraspecific competition. Contractions are especially apparent today because many overexploited fish populations are at historically low levels of abundance (Food and Agriculture Organization of the United Nations 2002).

DDHS has implications for management (Winters and Wheeler 1985; Swain and Sinclair 1994; Hutchings 1996). Catchability, the proportion of a population captured per unit effort, is influenced by changes in distribution (Paloheimo and Dickie 1964; Winters and Wheeler 1985) and catchability can remain stable or increase as population size decreases (Hutchings 1996). Thus, commercial catch data can

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provide a very poor index of the true abundance of a population (Walters and Maguire 1996). The weakness of the relationship between catchability and population abundance results from the nonrandom searching behaviour of fishers, a characteristic also associated with the behaviour of many predators. Thus, once a population contracts, it becomes increasingly vulnerable to fishing and predation pressure and this may inhibit it from recovering over time scales that would otherwise be expected.

In empirical studies of DDHS, optimal habitat is usually inferred as the area into which a population contracts at low abundance (Myers and Stokes 1989; Marshall and Frank 1995). Most studies of DDHS have focused on prey availability as the primary or sole factor influencing habitat selection without necessarily quantifying habitat quality beforehand. In practice, it is difficult to measure prey availability and distribution along with the physical habitat characteristics (temperature, depth, and substrate) and other ecological factors (predators) that may influence suitability (MacCall 1990). Moreover, delimiting the spatial extent and abundance of prey resources for widespread demersal species is complicated by the generalist nature of their diets and prey switching behaviour (Link and Garrison 2002).

Theoretical methods have been developed to predict how DDHS may operate with respect to habitat suitability and population size. One of the simplest approaches is the Fretwell and Lucas (1970) ideal free distribution (IFD) theory, which assumes that, at equilibrium, all individuals are free to move, will move to areas that will maximize their fitness, and are equal in terms of competitive ability and achieved fitness. MacCall (1990) applied IFD theory to a pelagic fish population in the form of a basin model and demonstrated how geographical range contraction and expansion could result from DDHS.

By definition, the concept of habitat suitability must somehow relate to fitness or proxies of fitness, which include reproductive success, intake rate, and growth rate (Fretwell and Lucas 1970). Fitness is not easy to measure at large scales in the marine environment, but growth rate can be linked to temperature and prey availability (Hughes and Grand 2000). Older and larger cod have lower optimal temperatures for growth (Björnsson and Steinarsson 2002) and the local distribution of cod is also known to depend on depth and temperature (Ottersen et al. 1998; Swain 1999).

Since growth rate – temperature relationships derived from experimental studies are independent of survey data, their incorporation into an IFD model provides a basis for predicting distribution–abundance relationships. Here, we test for distribution–abundance relationships for juvenile (ages-1 and -2) North Sea Atlantic cod (*Gadus morhua*) and test whether theoretical predictions based on IFD and optimal growth temperatures are consistent with survey observations. Our expectation is that cod will be found in habitats that optimize potential growth at low abundance but will spread to less favourable habitat as abundance increases. We make the following simplifying assumptions: that cod behaviour is ideal free and that cod maximize growth rates (and fitness) in response to their environment (in this case temperature). A validated model of the distribution–abundance relationship is a first step towards defining areas where juvenile cod may be vulnerable to fishing or predation, identifying closed areas that may reduce juvenile bycatch mortality and for predicting the impacts of climate variation and change.

Methods

Survey data

Juvenile cod relative abundance indices and bottom temperatures were determined from data collected during the third quarter (August–September) English groundfish survey. This survey was conducted annually from 1977 to 2002 by the research vessel *Cirolana*. From 1977 to 1981, three replicate tows were completed in a smaller number of International Council for the Exploration of the Sea (ICES) rectangles (boxes of 1° longitude × 0.5° latitude) (Fig. 1). From 1982, single tows were completed in a greater number of rectangles (Fig. 1). From 1977 to 1990, a Granton trawl was used and trawl durations were approximately 60 min along the bottom, and since 1991, a Grande Ouverture Verticale trawl has been used with trawl durations lasting approximately 30 min along the bottom. Since comparisons between relative density and distributional area were made between survey observations and model predictions within each year, these gear changes should not have an appreciable effect on our analyses. Relative survey catch density was expressed as mean numbers per standardized tow (per hour) for each ICES rectangle. An index of the total abundance of juvenile cod in the North Sea was obtained from numbers at age in the most recent virtual population analysis (VPA) (ICES 2004) for ICES areas IV, VIId, and part of IIIa (North Sea, English Channel, and Skagerrak). The spatial area, however, used for the relative abundance indices for this assessment is mainly based on ICES area IV (North Sea) and corresponds closely to the spatial coverage of the English groundfish survey.

Mean bottom temperatures were obtained from conductivity, temperature, and depth data collected on the third quarter (August–September) English groundfish survey. Mean temperatures for each station were reported for all years except 1999, when temperature data were not available for most of the survey area. The mean near-bottom temperatures were calculated for the duration that the trawl was in contact with the seabed in each tow.

Empirical distribution–abundance relationships

If distribution changes with total abundance, the area occupied by the population should either increase (expansion) or decrease (contraction) with total population abundance (Myers and Stokes 1989; Swain and Sinclair 1994; Marshall and Frank 1995). This study was limited to ages-1 and -2 cod owing to the higher abundance and hence greater statistical power of surveys in detecting distribution of these ages compared with older ages, which experience higher fishing mortality. Furthermore, distributions of older ages may be influenced by the spatial allocation of fishing effort, something we do not take account of in this study. To test for distribution–abundance relationships for ages-1 and -2 cod, we measured the area occupied by the population in each year as the minimum number of ICES rectangles that con-

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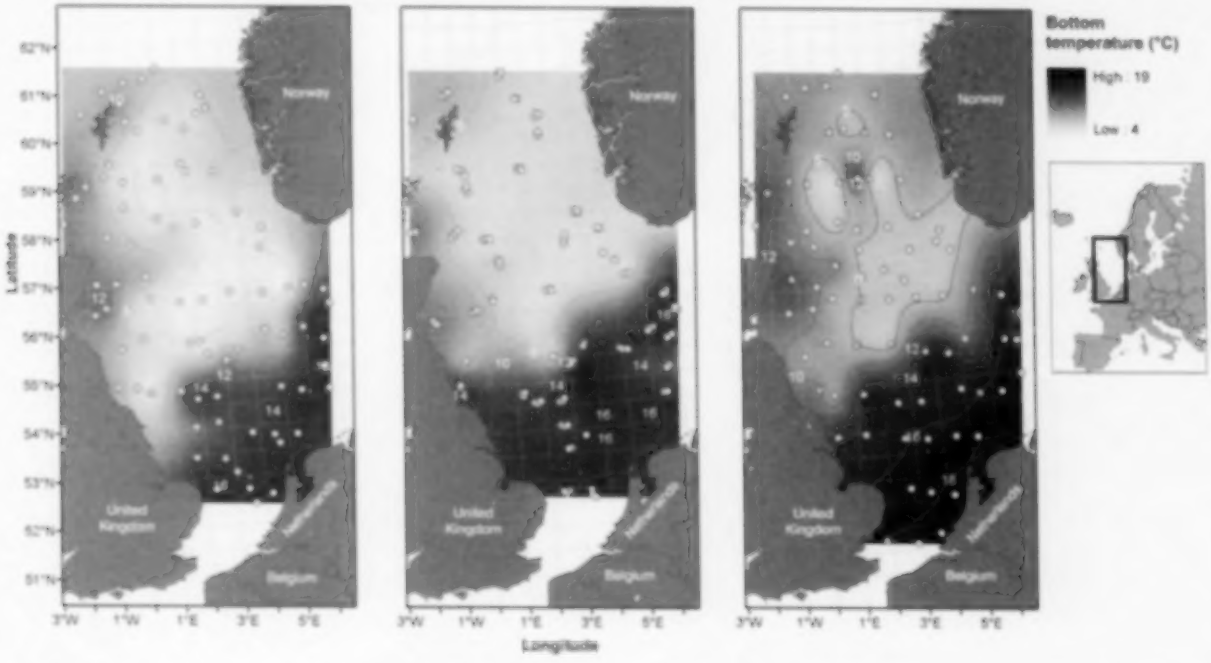
Fig. 1. Positions of survey locations in the North Sea (circles) during English groundfish surveys in three years, 1977, 1986, and 1998. Shaded contours show the cubic spline surface for bottom temperature based on measurements at survey positions. Bottom temperatures were extracted from the cubic spline predictions and assigned to ICES rectangles (grid cells).



tained >95% of the total relative abundance as estimated from the cumulative distribution of relative survey catch densities. This was then expressed as a proportion of the total number of rectangles surveyed in each year to account for the fact that the spatial coverage of the survey during 1977–1981 was less than in the later part of the time series and to account for differences in spatial coverage from year to year (some stations are not sampled every year owing to poor weather or equipment failure). This approach avoided possible inflation of the correlation between stock area and population size owing to the scaling of local density and population size, even in the absence of a distribution–abundance relationship (Swain and Sinclair 1994; Fisher and Frank 2004).

IFD model description

The IFD of individuals can be defined as the distribution of animals at equilibrium to areas of equal and maximum realized suitabilities (Fretwell and Lucas 1970). Because underlying habitat suitability is positively related to fitness but the increasing density of individuals decreases fitness, individuals spread into less preferred habitats as the local density increases. According to the IFD model, individuals ultimately settle into the areas that allow them to achieve equal and maximum fitness (realized suitability). Based on the model, the realized suitability will be equal to the basic suitability only when the number of individuals present is equal to zero. As the number of individuals increases, the realized suitability becomes lower than the basic habitat suitability. The effect of density-dependent realized suitability can be specified as a negative linear relationship with a constant slope (1) as in MacCall (1990):

$$(1) \qquad r_i = B_i(1 - kN_i)$$

where r_i equals the maximum realized suitability of animals in site i, B_i is the basic habitat suitability at location i, N_i is local population number per unit area, and k is the density-dependent coefficient or rate at which individuals spread into less preferred habitats as local density increases. The total population size N must equal the sum of all N_i from $i = 1$ to n, where n is the total number of locations. According to eq. 1, the carrying capacity of the habitat is inversely related to the k parameter ($K = 1/k$), where K is defined as the total population size at which $r_i = 0$ in all habitats (MacCall 1990).

Nonlinear forms of eq. 1 are also possible, but the shape of nonlinear relationships is difficult to justify a priori. Furthermore, MacCall (1990) showed that linear, concave, and convex families of these relationships give very similar equilibrium solutions. We thus limited our model to the linear fixed K relationship of MacCall (1990).

In the IFD, only habitats that allow individuals to achieve maximum and equal fitness are occupied. To determine the maximum (r^*) across all habitats, the model is run iteratively ($t = 1, 2, \ldots, n$), where n is reached when $\partial r/\partial t = 0$ to obtain the equilibrium solution of r^*. For each population size, all habitats where $B > r^*$ are occupied. The model predicts that at low population size, individuals only occupy areas with the very highest habitat suitability, at medium population size, they spread into less favourable habitat, and

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when the total population size equals the carrying capacity, all habitats are occupied (MacCall 1990).

Model application

The IFD was used to model the distribution of juvenile cod (ages 1 and 2) during August of each year. August was chosen owing to the longer time series of the English groundfish survey available at that time of year. It is reasonable to assume that growth rates would be maximized during the summer feeding season compared with other times of year. Assessment of habitat suitability required identification of physical components of the habitat that are linked to fitness. Lacking long-term data sets on parameters such as prey availability, habitat suitability was estimated from knowledge of optimal temperature for growth and recorded bottom temperatures.

To relate the expansion and contraction of cod stocks over the entire North Sea to suitability, we required bottom temperature data for the entire North Sea. Since sea bottom temperatures from the English third quarter ground fish survey between the years 1977 and 2002 covered the majority of the North Sea (3.835°W to 7.35°E and 61.532°N to 51.60°N), we used an interpolation technique to estimate temperatures for all parts of the model grid that did not contain survey observations. The gradual changes in bottom temperature data over a horizontal plane suggested that a spline technique was suitable for interpolation (Johnson et al. 2001). Comparison of predictions based on a regular spline (cubic) and tension spline technique (Johnson et al. 2001) demonstrated that the tension spline provided the more accurate representation. Thus, the tension spline was used to estimate bottom temperature at the centre of each ICES rectangle (area of rectangle 3720 km^2 at 53°N) in ICES divisions IVa and IVb. Bottom temperatures were not recorded anywhere in the Norwegian Deep, but interpolated temperatures were consistent with those reported by Janssen et al. (1999). The distribution of bottom temperature is shown in three years when the overall mean bottom temperature during August was the coldest (1986), intermediate (1977), and warmest (1998) (Fig. 1).

Habitat suitability was determined from the optimal temperature for specific growth rate of juvenile cod based on experimental studies reported in Björnsson and Steinarsson (2002) and values were rescaled between 0 and 1:

$$(2) \qquad B_i = 0.5735\, T_i\, W^{(-0.1934-0.02001\, T_i)}$$

where B_i is the habitat suitability in rectangle i, T_i is the bottom temperature in rectangle i, and W is the mean weight of cod. Mean weights of age-1 and age-2 cod were calculated as 247 and 817 g, respectively, using pooled weight-at-age data for the entire survey period for years when individual weights were collected. These mean weights were assumed to be stable across years and across different areas of the North Sea. Interannual differences in weight were not significant for age-1 cod ($p > 0.5$) but were significant for age-2 cod ($p < 0.001$) despite the large degree of variability in observed individual weights within years. A preliminary analysis of the effect of using the extreme upper and lower weights for both ages had a negligible effect on the model results; therefore, mean values based on the pooled data were used for each age. Although latitudinal differences in cod growth rates have been shown for larger, older cod across stocks (Brander 1995), no latitudinal pattern in mean weight was apparent for ages-1 and -2 cod collected on the English groundfish survey in the North Sea. Using eq. 2, the optimal temperatures were 9.1 °C for age-1 cod and 7.4 °C for age-2 cod. Highly suitable habitat (above or equal to 0.99 on a scale of 0 to 1) for age-1 cod approximately corresponds to the areas between the 8 and 10 °C contours, whereas for age-2 cod, it corresponds to areas bounded by the 6 to 8 °C contours (as seen in Fig. 1).

VPA estimates (numbers of age-1 cod) were used as the model input for the total abundance of individuals in each year. The VPA estimates are not entirely independent of the survey data, since these data, along with data collected by other countries and from the commercial fishery, contribute to the VPA calibration procedure. It should be noted that these VPA estimates may be underestimates in recent years owing to discarding (ICES 2004).

In reality, estimating the rate of spread across habitats as the population size increases (or density-dependent coefficient k) is difficult, since k can be interpreted as the inverse of the carrying capacity (MacCall 1990) and K is unlikely to remain constant under changing ecological conditions (Krebs 2002). Differences in carrying capacity among stocks can be considerable. Thus, Myers et al. (2001) showed that the variability in relative carrying capacity of cod stocks, as derived from Beverton–Holt stock–recruitment relationships (standardized for stock area), was 20-fold in the North Atlantic and that carrying capacity was negatively correlated with temperature. However, such estimates of carrying capacity do not necessarily represent the carrying capacity of a habitat. In eq. (1), K represents a reference value for the population that fills the habitat to the point of neutral fitness.

Given the preceding constraints, the maximum historic abundance of age-1 cod reported in the VPA was used as the reference value K. This value corresponded to the abundant 1970 year-class that was observed as 1-year-olds during 1971. We ran the model for K values from 1K to 20K, thus encompassing the degree of variability in K derived from standardized stock–recruit relationships across the North Atlantic (Myers et al. 2001).

Age-2 cod are subject to significant fishing mortality. We estimated the reference value for age-2 cod (K_2) in the absence of fishing using the best-fitted parameter estimate for age-1 cod (K_1):

$$(3) \qquad K_2 = K_1 e^{-M}$$

where natural mortality (M) = 0.35·year^{-1} from multispecies VPA estimates of M for age-2 cod (ICES 2004).

Comparison of model with data

Model output was compared with survey data by examining relationships between the minimum area occupied by 95% of the stock (expressed as a proportion) and the total population abundance (N) in each year. A comparison of survey catch densities and the habitat suitability index (by ICES rectangle) was used to evaluate whether the highest densities were observed in the most suitable habitats. For each year, the model was run for 10 different values of k and the predicted values that best fit the observed data were determined by the minimum sum of squares between the

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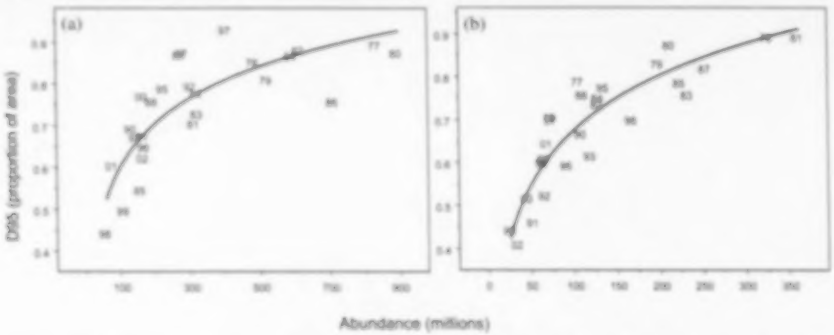
Fig. 2. Proportion of area occupied by 95% of (*a*) age-1 and (*b*) age-2 Atlantic cod (*Gadus morhua*). Plotted characters indicate the last two digits of the year that observations were made. Lines indicate least squares fit.



model and observations. To compare survey observations and model predictions that incorporated DDHS in each year, we compared plots of the mean observed numbers per ICES rectangle (survey) with the mean predicted numbers per ICES rectangle (model) with respect to the habitat suitability. Both indices were expressed as proportions of the sum of these numbers across all rectangles sampled, to standardize to a common scale. We examined overall trends by summing the proportions of total density into discrete 0.01 intervals of habitat suitability, thus constructing a coarser density distribution as a function of habitat suitability (*x* axis) for both the survey and model values in each year. This allowed us to compare model and the survey observations despite the low variability in the modelled observations relative to the survey observations. Spearman's rank correlation was used to test for significant correlations between the modelled (predicted) and observed distributions. A two-sample Kolmogorov–Smirnov test was used to test the relationship between the two distributions and to test the null hypothesis that the model and survey distribution functions were equal.

Results

Empirical distribution–abundance relationships

There were significant positive relationships between the minimum area occupied by 95% of age-1 and age-2 cod and corresponding indices of total abundance. The relationships between D (proportion of area) and N (number in millions) were clearly curvilinear for age-1 and age-2 cod (Fig. 2) and the following regression model was fit to the data:

$$(4) \qquad D = \beta_0 + \beta_1 \ln(N)$$

The resulting regression fits were highly significant (age 1: $R^2 = 0.67$, $p < 0.001$; age 2: $R^2 = 0.82$, $p < 0.001$). The parameters were $\beta_0 = -0.074$ and $\beta_1 = 0.1471$ for age-1 cod and $\beta_0 = -0.1695$ and $\beta_1 = 0.1835$ for age-2 cod.

Comparison of data with theoretical model

In all years except 1991, the best fit was obtained by the value of k that was calculated as the inverse of the maximum observed historic numbers of age-1 cod. In 1991, there was a slightly better fit to a k value calculated as the inverse of two times the maximum observed numbers. Subsequent comparisons of model and observations were based on these best fitting values of k.

Comparison of the model and survey data showed that there was a similar curvilinear relationship between proportion of area occupied by the stock (D) and total abundance (N). The model produced D95 values that were higher than the observed values but the maximum D95 values occurred at similar total abundance (approximately 200 million individuals).

Age-1 and age-2 cod showed similar responses to changes in abundance. Both model and survey observations showed that in low abundance years, the highest relative catch densities occurred at the highest habitat suitabilities and, in high abundance years, that high densities spread across the entire area (Figs. 3 and 4). There was much more variability in survey data than model predictions, consistent with the patchy nature of fish distributions that may be driven by local habitat heterogeneity, prey availability, and predator distributions (e.g., Hughes 1993). Results from the statistical comparison of the survey versus model distributions of the proportions of total density across 0.01 intervals of habitat suitability revealed consistent patterns between the two. In the majority of the 25 years (exceptions were 1983, 1992, and 1995 for age-1 cod and 1978, 1981, 1983, 1987, and 1998 for age-2 cod), the modelled and observed distributions were equal (Kolmogorov–Smirnov two-sample test, null hypothesis not rejected when $p > 0.05$). The Spearman rank statistic revealed a consistently significant agreement in most years (Table 1).

There were slight changes in the range of the habitat suitability index over time, and these changes could be interpreted as changes in the amount of optimal habitat available. The 5% quantiles of the ranked habitat suitability indices (poorest to best) of the ICES rectangles that were sampled in all 25 years were calculated for age-1 and age-2 cod for each year (Fig. 5). These values represent the lower threshold of 95% of the available habitat each year. The index declined slightly over time (Fig. 5) and was lower for age-2 than for age-1 cod. However, on a suitability scale from 0 to 1, all lower thresholds represent habitat that is still of reasonable quality for cod growth.

Discussion

The reported positive curvilinear relationship between the distribution and abundance of juvenile North Sea cod is con-

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Fig. 3. Observed (open circles) and predicted (broken lines) proportion of total age-1 Atlantic cod (*Gadus morhua*) density on an ICES rectangle basis for the two highest abundance years, (*a*) 1977 and (*b*) 1980, and two lowest abundance years, (*c*) 1998 and (*d*) 2001, used in the analysis. The total number of rectangles for which observations were available (each data point) varied across years. In 1977 and 1980, 48 and 49 rectangles were sampled, respectively, and the total number of tows each year, including replicates, was 110 and 115. In 1998 and 2001, 74 rectangles were sampled, with one tow in each.

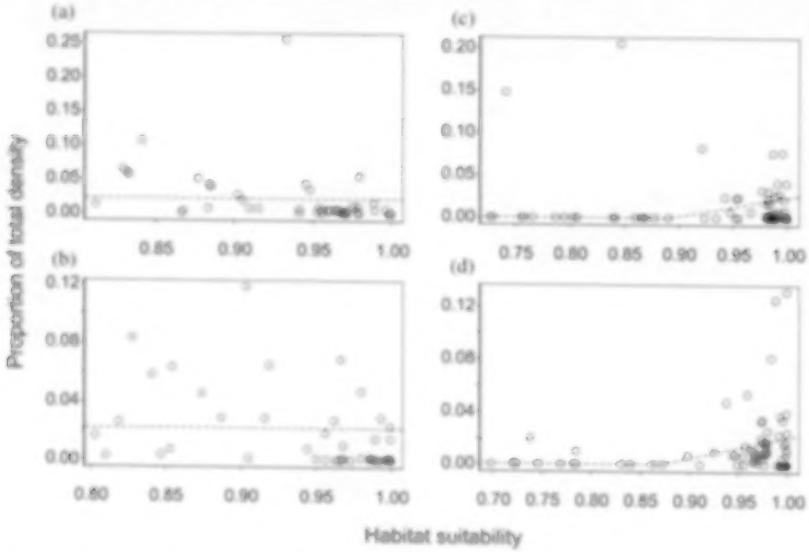
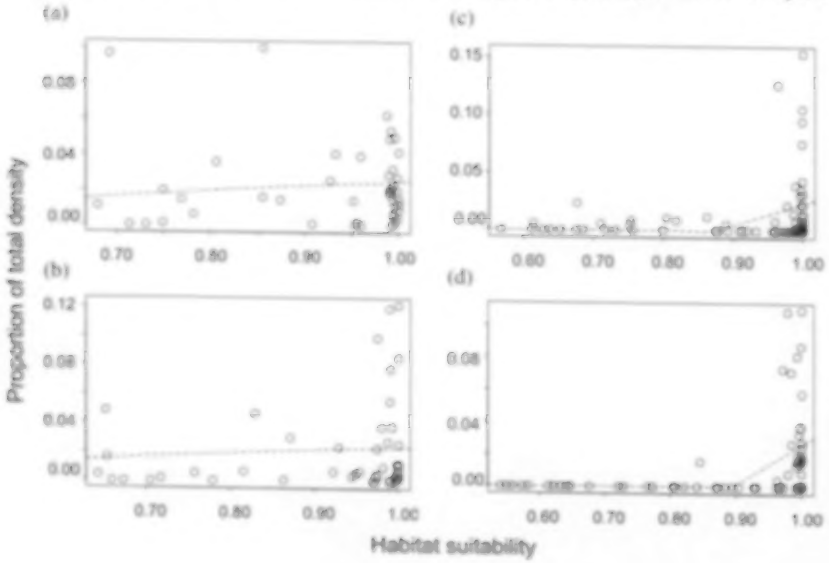


Fig. 4. Observed (open circles) and predicted (broken lines) proportion of total age-2 Atlantic cod (*Gadus morhua*) density on an ICES rectangle basis for the two highest abundance years, (*a*) 1978 and (*b*) 1981, and two lowest abundance years (*c*) 2001 and (*d*) 2002, used in the analysis. In 1978 and 1981, 48 rectangles were sampled and the total number of tows each year, including replicates, was 113 and 114, respectively. In 2001 and 2002, 74 and 75 rectangles were sampled, respectively, with one tow per rectangle.



sistent with the patterns observed for other cod stocks (Swain and Wade 1993; Swain and Sinclair 1994; Hutchings 1996). We found striking similarities between survey abundance estimates and predictions based on a simple IFD DDHS model (Fretwell and Lucas 1970; MacCall 1990). Our measure of habitat suitability was based on one factor as a proxy for fitness: optimal temperature for growth based on the experimentally derived relationship of Björnsson and Steinarsson (2002). Since the relationship depended on size as well as temperature, we carried out the analysis for 1- and 2-year-old cod. Distribution–abundance relationships were consistent across years and, in medium- to low-abundance

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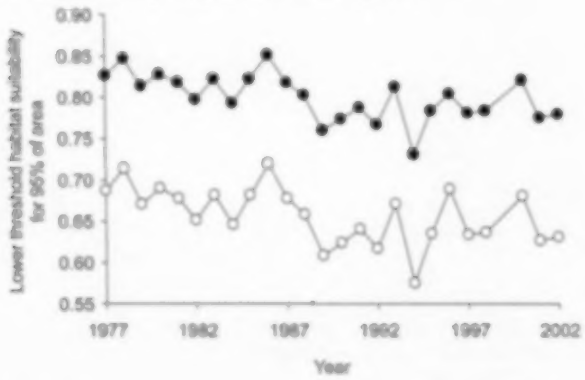
Table 1. Spearman rank correlation coefficient (r_s) and Kolmogorov–Smirnov two-sample goodness-of-fit test statistic (KS) for the null hypothesis that observed (survey) versus predicted (IFD model) distributions are equal.

	Age 1				Age 2			
Year	r_s	p	KS	p	r_s	p	KS	p
1977	0.271	0.297	0.375	**0.215**	0.267	0.382	0.250	**0.869**
1978	0.666	**0.017**	0.286	**0.635**	0.606	**0.024**	0.400	0.184
1979	0.138	0.571	0.444	**0.056**	0.922	**0.001**	0.231	**0.898**
1980	0.400	0.136	0.333	**0.386**	0.674	**0.009**	0.250	**0.716**
1981	0.693	**0.007**	0.438	**0.093**	0.761	**0.005**	0.600	0.008
1982	0.899	**0.001**	0.286	**0.635**	0.749	**0.003**	0.235	**0.751**
1983	0.769	**0.002**	0.471	0.045	0.312	0.187	0.526	0.009
1984	0.775	**0.002**	0.176	**0.963**	0.628	**0.015**	0.375	**0.215**
1985	0.663	**0.008**	0.235	**0.751**	0.593	**0.015**	0.444	**0.056**
1986	0.472	**0.046**	0.316	**0.306**	0.832	**<0.001**	0.143	**0.987**
1987	0.309	0.191	0.421	**0.068**	0.569	**0.013**	0.450	0.034
1988	0.500	0.062	0.333	**0.386**	0.467	0.072	0.250	**0.716**
1989	0.782	**0.001**	0.421	**0.068**	0.432	0.075	0.167	**0.972**
1990	0.608	**0.008**	0.150	**0.983**	0.356	0.132	0.211	**0.808**
1991	0.374	0.087	0.182	**0.872**	0.570	**0.013**	0.150	**0.983**
1992	0.707	**0.002**	0.571	0.002	0.645	**0.008**	0.167	**0.972**
1993	0.826	**<0.001**	0.250	**0.571**	0.733	**0.005**	0.438	**0.093**
1994	0.961	**<0.001**	0.333	**0.386**	0.665	**0.017**	0.214	**0.921**
1995	0.669	**0.006**	0.500	0.021	0.662	**0.014**	0.400	**0.184**
1996	0.682	**0.002**	0.136	**0.990**	0.778	**0.001**	0.333	**0.275**
1997	0.668	**0.013**	0.200	**0.938**	0.506	0.051	0.250	**0.716**
1998	0.661	**0.004**	0.250	**0.571**	0.606	**0.007**	0.571	0.002
2000	0.751	**0.002**	0.263	**0.538**	0.335	0.156	0.105	**1.000**
2001	0.806	**0.001**	0.167	**0.972**	0.678	**0.011**	0.267	**0.678**
2002	0.335	0.156	0.263	**0.538**	0.701	**0.005**	0.118	**1.000**

Note: Associated p values are shown for tests on age-1 and age-2 Atlantic cod (*Gadus morhua*) each year (1977–2002). Significant goodness-of-fit ($p > 0.05$) and correlations ($p < 0.05$) are in bold.

Fig. 5. Lower 5% quantiles of habitat suitability for each year representing 95% of the total area falling above this level for age-1 (solid circles) and age-2 (open circles) Atlantic cod (*Gadus morhua*) over 1977–2002. The total habitat area was fixed and encompassed the spatial extent of the survey common to all years (comprising 135 ICES rectangles).



years, highest observed catch densities were located in areas of highest habitat suitability. Furthermore, the IFD model predictions that included the effect of DDHS were consistent with the survey observations over the entire range of population sizes observed. DDHS may explain the spreading into suboptimal habitat owing to crowding and increased intraspecific competition for resources at high densities.

IFD theory assumes that all individuals are free to move between habitats. The potential for free movement could be influenced by substructuring of the cod population, and there is some evidence from microsatellite analysis and tagging that there are genetically identifiable spawning subpopulations in the North Sea (Hutchinson et al. 2001). The extent to which their progeny mix and are "ideal free" in their behaviour is not known and could most usefully be ascertained by tagging.

An index of habitat suitability based on temperature provides a simple index of habitat preference for juvenile cod. The advantages of using temperature are that growth–temperature relationships are available and that temperatures are widely measured at large spatial scales in the marine environment. Other factors such as prey abundance, predation risk, substrate type, water column stratification, depth, and salinity are also likely to influence suitability (e.g., Lindholm et al. 1999), but it is usually unrealistic to measure these and their associations with fitness at large spatial scales in the marine environment. Moreover, many of these factors probably influence distribution at much smaller scales than temperature and the density estimates from trawl tows will tend to integrate small-scale patchiness.

The temperature–growth model developed by Björnsson and Steinarsson (2002) was based on an experimental study

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using Icelandic cod where high-quality food was unlimited. If the shape of the relationship changes in response to resource availability in the North Sea or there are genetic differences in metabolic efficiency between stocks, then this will affect the index of habitat suitability. Brander (2003) proposed an alternative growth temperature model based on assessment weight-at-age data. While this model may better describe the optimum growth temperatures for older fish in the North Sea, it cannot reliably be compared with that of Björnsson and Steinarsson (2002) because gear selectivity may bias growth estimates for the younger age classes in assessment weight at age data.

The spawning stock biomass of North Sea cod has declined to historically low levels in recent years and the abundance of 1- and 2-year-old fish has also fallen owing to poor recruitment and fishing mortality (O'Brien et al. 2000; ICES 2003). Our results showed that when the abundance of 1- and 2-year-old cod was low, there was a contraction of the stock into areas with a habitat suitability index >0.90. This resulted in the spatial extent covered by 95% of age-1 and age-2 cod falling to less than half of that available during years of very low abundance (1991, 2000, and 2002 for age-1 cod and 1985 and 1998 for age-2 cod). The location of these "basins" of good habitat seemed to persist across years, namely the northeastern North Sea for age-1 cod and the north-central North Sea for age-2 cod, although the size of the area was variable. Locations that had highly variable habitat suitability appeared to be associated with changes in the position of steep thermal gradients. The vulnerability of young cod to fishing or predation at low population size could be exacerbated by the effect of this relationship. First, the areas of high suitability for age-1 cod broadly correspond to areas where international demersal fishing effort is also high (Jennings et al. 1999). Thus, the relative catchability of young cod (as bycatches and in directed fisheries) may increase during periods of low abundance, further curtailing recruitment to the adult stock. This hypothesis would need to be tested by a careful analysis of catch and bycatch rates in relation to local abundance in areas of high and low habitat suitability. Second, young cod may become more vulnerable to predators when they are confined to small areas of high habitat suitability. For example, the distribution–abundance relationship for Newfoundland cod may have led to a predator pit (Hilborn and Walters 1992), which may be inhibiting the recovery of the stock after a fishery closure spanning more than a decade.

Knowledge of the distribution–abundance relationship for juvenile cod and the recognition that temperature is potentially a good predictor of areas of high suitability may assist with management. Thus, temperature could be used to define areas of high suitability where small-mesh fisheries might be restricted to reduce bycatch mortality or to define areas for real time and area closures when abundance is unusually low. The corollary of not including the habitat of highest suitability inside such closed or regulated areas is that fishing effort might be forced to relocate to areas where suitability and catchability are higher (Jennings 2001).

The recent dramatic decline in stock biomass of North Sea cod has been linked to a combination of overfishing and climate change affecting recruitment (O'Brien et al. 2000; Beaugrand et al. 2003). However, the biological mechanisms behind the environmental link remain obscure. Temperatures in the period February–June during the 1st year of life appear to have the most impact on recruitment, implying that the early life history stages (0-group) are affected (Planque and Frédou 1999). Over the last 40 years, average North Sea temperatures have increased, but even in recent years, the majority of the North Sea remains a suitable habitat for growth, at least according to the temperature-driven DDHS model. Current climate predictions show a steady warming trend in North Sea temperatures over the next 50 years (Clark et al. 2003), so the area of good habitat for immature cod growth is likely to continue to slowly decline. If cod stock abundance remains low, this will probably lead to further contraction of the area in which immature cod are found and further increase their potential vulnerability to fishing and predation.

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Foraging behavior, morphology, and life history variation determine the ontogeny of piscivory in two closely related predators

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Abstract: The ontogeny of piscivory is an important process during the early life history of many fishes, and why ontogenetic patterns of closely related species vary is unclear. We experimentally evaluated the importance of several factors that can determine the switch to piscivory in two predators with different dietary ontogenies: walleye (*Sander vitreus*), a specialist piscivore, and yellow perch (*Perca flavescens*), a dietary generalist. We conducted growth and prey selection experiments across several sizes of both predators using fish, zooplankton, and benthic invertebrates as prey. Walleye exhibited piscivorous feeding behavior throughout all size classes (20–80 mm), whereas yellow perch were generalist predators, showing negative to neutral selection for fish prey and lower growth than walleye when feeding on fish. Walleye foraged more efficiently than yellow perch on all prey types, in part because gape widths of walleye increased more quickly with size. Bioenergetic model simulations showed that walleye grew slower than yellow perch when the proportion of fish was low in diets, but walleye growth was faster than yellow perch on a diet dominated by fish. Feeding behavior, morphology, and life history patterns likely work in combination, allowing walleye to switch to piscivory earlier than yellow perch.

Résumé : L'ontogenèse de l'ichtyophagie est une étape importante du cycle biologique de plusieurs jeunes poissons et il n'est pas clair pourquoi les patrons ontogéniques d'espèces apparentées peuvent différer. Nous avons évalué expérimentalement l'importance de plusieurs facteurs qui peuvent favoriser le passage à l'ichtyophagie chez deux prédateurs qui possèdent des ontogenèses alimentaires différentes, le doré jaune (*Sander vitreus*), un piscivore spécialisé, et la perchaude (*Perca flavescens*), un généraliste alimentaire. Nous avons mené des expériences de croissance et de sélection de proies chez diverses tailles des deux prédateurs et utilisé des poissons, du zooplancton et des invertébrés benthiques comme proies. Les dorés ont un comportement alimentaire de piscivores à toutes les tailles (20–80 mm), alors que les perchaudes sont des prédateurs généralistes qui font une sélection négative ou neutre des poissons comme proies; les perchaudes croissent aussi moins rapidement que les dorés lorsqu'ils s'alimentent de poissons. Les dorés sont plus efficaces que les perchaudes pour attraper tous les types de proies, en partie parce que la largeur de l'ouverture de leur bouche croît plus rapidement en fonction de la taille. Des simulations d'un modèle bioénergétique montrent que les dorés croissent plus lentement que les perchaudes lorsque la proportion de poissons dans leur régime alimentaire est faible, mais que les dorés grandissent plus rapidement que les perchaudes lorsque le régime est dominé par les poissons. Le comportement alimentaire, la morphologie et les patterns du cycle biologique concourent tous vraisemblablement à permettre au doré de faire le passage vers l'ichtyophagie plus tôt que la perchaude.

[Traduit par la Rédaction]

Introduction

Many organisms undergo ontogenetic diet shifts during early life-history stages. These shifts often have large implications for individuals, populations, and communities (Werner and Gilliam 1984; Mittelbach and Persson 1998). For many fishes, diet shifts eventually result in piscivory (e.g., yellow perch, *Perca flavescens*, Fullhart et al. 2002; walleye, *Sander vitreus*, Mathias and Li 1982; largemouth bass, *Micropterus salmoides*, Olson 1996). This switch to piscivory is viewed as favorable to individuals because of the associated increase in growth and survival (Ludsin and DeVries 1997; Persson and Brönmark 2002). Furthermore, the timing and extent of switching to piscivory can influence

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the recruitment of cohorts within a population (Olson 1996). Piscivory can also be important in structuring communities by decreasing the abundance of prey fish available to other predators, or by reducing intraspecific competition and increasing the growth of prey populations (Tonn et al. 1991). Thus, understanding the mechanisms driving the onset of ontogeny of piscivory will improve our understanding of several ecological processes.

Young fish that switch to a piscivorous diet often realize a substantial increase in growth and body condition compared with other fish within a cohort that maintain a diet of invertebrate prey (Keast and McA. Eadie 1985; Buijse and Houthuijzen 1992). Further, these larger (piscivorous) fish experience higher over-winter survival and recruitment compared with smaller (non-piscivorous) cohort members (Ludsin and De Vries 1997). Although the advantages of switching to a piscivorous diet are well documented, little is known either about the mechanisms determining the ontogeny of piscivory in fishes, or why the timing of switching to piscivory among fish species is so variable.

Despite the benefits of switching to a fish diet, the size at which fish switch to piscivory varies widely, with some species switching when they are as small as 30 mm (e.g., walleye), while others can take up to 3 years (e.g., yellow perch). These early and late switching groups have been called "specialists" and "secondary" piscivores (Keast 1985). Optimal foraging theory (e.g., Werner and Hall 1974) suggests that early switching piscivores achieve faster growth because they derive higher energetic returns from fish prey than alternative prey such as invertebrates. Furthermore, some specialist piscivores actively choose fish prey, as opposed to invertebrate prey, because fish prey maximizes net energy intake per time spent foraging, as predicted by optimal foraging theory (Einfalt and Wahl 1997; Turesson et al. 2002). In contrast, secondary piscivores may passively choose prey items based on foraging efficiency and encounter rates (Turesson et al. 2002). These predators may still consume fish prey, but do not actively choose this prey type. Based on optimal foraging considerations, young secondary piscivores are likely to grow best on invertebrate diets, even though they can select fish prey.

To gain insight into the variability in the extent and timing of switching to piscivory, Mittelbach and Persson (1998) related several variables (e.g., spawning date, prey manipulation times, and morphology) among fish species in the northern hemisphere to the ontogeny of piscivory. They concluded that much of the variation in size at switching could be attributed to differences in predator morphology, especially predator gape size, although they did not explicitly explore their results in the context of optimal foraging theory. Larger gape widths may allow early switching piscivores to actively choose fish because they can more efficiently consume fish prey, thus maximizing energy intake. However, Mittelbach and Persson (1998) cautioned that much of the data used in their analysis were focused on age-1 and older fishes. As such, their conclusions may not apply to younger ages, when many fishes switch to piscivory. They recommended comparing age-0 morphology, growth, foraging efficiency, and life history variation of specialist and secondary piscivores to further understand the mechanisms determining the ontogeny of piscivory.

Walleye and yellow perch provide good models to examine the predictions of Mittelbach and Persson (1998) because they are closely related (two genera in the family Percidae), co-occur in many systems, and are piscivorous as adults, yet differ markedly in their timing of switch to piscivory. Diets of age-0 walleye as small as 35 mm are dominated by fish (Mathias and Li 1982); they are considered specialist piscivores. In contrast, yellow perch generally do not consume fish prey until age 1+ at lengths greater than 150 mm; typically they are not primarily piscivorous until age 4+ (Keast 1985; Fullhart et al. 2002). Walleye hatch earlier and at larger sizes (6–8 mm; Marshal 1977) than yellow perch (5–6 mm; Thorpe 1977), presumably leading to a size advantage over their prey (later hatching larval fish); they also possess canine teeth that aid in the grasping of prey (Craig 1987; Mittelbach and Persson 1998), whereas yellow perch lack canine teeth (Craig 1987). Differences among these characteristics may explain differences in the ontogeny of piscivory between walleye and yellow perch. We hypothesized that elements of optimal foraging theory could explain these observed differences in the timing of the switch to piscivory. We predicted the following: (*i*) walleye actively select fish over alternative prey because fish prey optimize caloric intake and growth, whereas yellow perch passively select prey based on foraging efficiency, with little change in growth among prey types; (*ii*) walleye gape size at a given length would be larger than that of yellow perch, allowing walleye to consume fish prey more efficiently than yellow perch; and (*iii*) life history differences favor the tendency of walleye to switch to piscivory at smaller sizes and realize faster growth rates than yellow perch maintaining a mixed diet; however, walleye, as a specialist piscivore, would grow slower than yellow perch if both predators could not switch to piscivory, despite an earlier hatching date.

We tested these predictions by quantifying several factors that may determine the ontogeny of piscivory of walleye and yellow perch. We first quantified prey selection patterns and growth rates of the two predators across several sizes to determine the size at which the switch to piscivory is complete, and if switching to a fish diet resulted in faster growth compared with alternate prey. We then experimentally quantified foraging behavior (capture efficiency and attack rate) to determine if either predator foraged more efficiently on fish prey. We also determined differences in mouth gapes for both predators to assess whether morphology contributed to the timing of diet changes. Finally, we compared the effects of variation in early life history (hatching date) and the ability to switch to piscivory (synchrony with prey fish appearance) or remain on a mixed diet (asynchrony with prey fish appearance) using bioenergetic model simulations. We predicted that growth would be similar for both predators when consuming the same diet despite differences in hatching date, but if either predator was able to shift to piscivory, they would realize faster growth than the other predator maintaining a mixed diet. This modeling approach allowed us to examine growth tradeoffs for both predators with differing hatching dates and a fish-dominated diet compared with a mixed diet. We modeled differing hatching dates, piscivorous and mixed diets, and the combination of hatching date and diet to determine how these two factors influence the growth of walleye and yellow perch. Specifically,

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we evaluated whether a specialist piscivore experienced cost in terms of decreased growth when it could not switch to piscivory, as compared with a generalist predator (Mittelbach and Persson 1998).

Materials and methods

To test our predictions, we designed a series of laboratory experiments to quantify size-specific growth, prey selection, and foraging efficiency of walleye and yellow perch. Each variable was quantified for both predators when offered fish and (or) two alternate invertebrate prey (zooplankton and benthic invertebrates), at two prey densities (high and low). We assessed whether prey selection could predict predator growth, and if so, we then determined whether the patterns could be explained by foraging behavior and morphology. All experiments were conducted during 1998–2001 at the Kaskaskia Biological Station (Illinois Natural History Survey, Sullivan, Illinois) using the same protocols, prey types, and observers. We conducted experiments on 20-, 40-, 60-, and 80-mm size classes of walleye and yellow perch. Predators used in each size class were ±2 mm of the target size (i.e., for the 20-mm size class, we used 18- to 22-mm predators). Age-0 bluegill (*Lepomis macrochirus*) were used as fish prey at 20%–25% of the total length of the predator throughout all experiments, except that larval common carp (*Cyprinus carpio*) were used for experiments with walleye in the 20-mm size class. Common carp and bluegill larvae have similar behavior and morphology at these sizes (Galarowicz and Wahl 2005). We used chironomid larvae as our benthic invertebrate prey (mean length = 11 mm, SE = 0.33), and daphnids as zooplankton prey (mean length = 1.7 mm, SE = 0.02). Walleye foraging and growth data were part of another study (see Galarowicz and Wahl (2005) for more details), but were combined with yellow perch data and reanalyzed here.

Prey selection

We quantified prey selection by observing a single predator foraging on prey items in rectangular (500 L, yellow perch) or circular (300–500 L, walleye) tanks. Although we used different arena sizes for the smaller size classes of walleye, prey densities were similar across all experiments. Fish, chironomids, and zooplankton prey were offered to the predators in two different combinations: (*i*) low fish (10·m^{-3}), high benthos (500·m^{-2}), and high zooplankton (25·L^{-1}), or (*ii*) high fish (100·m^{-3}), low benthos (25·m^{-2}), and low zooplankton (2.5·L^{-1}). We selected these combinations to offer the predators fish prey at both high and low densities relative to the other prey items. The prey densities used in our experiment represent high and low relative densities common in Illinois lakes (Welker et al. 1994; Claramunt and Wahl 2000). Prior to the start of each 30-min observation period, a single predator was acclimated in a small holding chamber within the behavioral arena for at least 30 min. During a trial, the number of strikes and captures for all prey items was recorded. Five replicate trials were conducted on both prey combinations offered to all size classes of yellow perch and walleye (n = 40 trials for each species).

To determine prey selectivity, we calculated mean Chesson's (1983) coefficient of selectivity (α) for each size class and prey density combination:

$$\alpha = \frac{r_i/n_i}{\sum_{i=1}^{m}(r_i/n_i)}$$

where r_i is the number of food type i in the predator diet, n_i is the number of food type i in the environment, and m is the number of prey types available. Mean alpha values (±95% confidence intervals) were compared against random feeding ($1/m$) to determine selectivity.

Growth

Specific growth rate was quantified for both length (to the nearest 0.5 mm) and mass (to the nearest 0.01 g) over a 7-day period of feeding on a diet of fish, zooplankton, or chironomids. A single juvenile walleye or yellow perch was placed in an aquarium (38 L) in an environmental chamber (20 °C, 12 h light – 12 h dark) and fed one prey type ad libitum. Prey (fish, chironomids, and daphnids) were added daily to each tank. Five to ten replicates were performed for each predator and prey-type combination. Specific growth rate (mm·mm^{-1}·day^{-1}, or g·g^{-1}·day^{-1}) was determined as the amount of length or weight gained, given the initial length or weight, over the experimental period. These data were analyzed using a three-factor (predator, prey, and predator size) analysis of variance (ANOVA); data were log-transformed, and means were separated using pair-wise comparisons and stepdown Bonferroni adjustments.

Foraging behavior

Behavioral experiments were conducted with all size classes of predators in 72-L aquaria. A single prey type was offered to the predators at both low (either 13 fish·m^{-3}, 50 benthic invertebrates·m^{-2}, or 2.5 zooplankton·L^{-1}) and high densities (either 66 fish·m^{-3}, 200 benthic invertebrates·m^{-2}, or 50 zooplankton·L^{-1}). We chose these prey densities to have sufficient prey to observe successful captures in a smaller arena while staying within prey range densities observed in Midwestern US lakes. A single predator was acclimated for at least 30 min in a partitioned section of the aquarium, and prey were allowed to acclimate in the remainder of the aquarium for at least 5 min prior to the start of each trial (see Galarowicz and Wahl (2005) for more details). Trials commenced when a predator was released from the enclosure by remotely lifting a partition. During each 15-min trial, we recorded the number of strikes, captures, and prey items consumed. At least five replicate trials were conducted on each size class and prey treatment combination. From these observations, we calculated capture efficiency (the number of strikes per capture), attack rates (the number of strikes per minute), and the total number of prey items consumed. All analyses were conducted using four-factor (predator, predator length, prey type, and prey density) ANOVA (data were log-transformed if non-normal). Means were separated using least-square means comparisons with stepdown Bonferroni adjustments.

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Morphology

Gape widths were estimated across a range of sizes (30–85 mm) for both predators (n = 20 walleye, n = 56 yellow perch). Gape was measured at the narrowest point inside the mouth of each predator using calipers. The relationship between gape width and predator total length was determined with linear regression. To prevent extrapolating beyond the range of our data, we restricted the intercept to the lower bounds of the data by subtracting the smallest length (30 mm) from all lengths. The slopes and intercepts of the regression lines were then compared using analysis of covariance (ANCOVA) to assess differences in gape across length.

Bioenergetic model simulations

We used the Wisconsin Bioenergetics Model 3.0 (Hanson et al. 1997) to estimate growth and consumption of the two predators under differing diets and hatch date conditions. We used the juvenile walleye and larval yellow perch submodels, as provided by Hanson et al. (1997), for all simulations. Temperature (°C) during 2002 at the 1-m depth interval in Lake Mendota, Wisconsin (Kratz 2002) was used to simulate temperature during a typical growing season from a system containing both walleye and yellow perch. Spawning occurred at 8.5 °C for walleye and 10 °C for yellow perch, the median values from ranges reported by Scott and Crossman (1973). Our simulations started at the commencement of exogenous feeding, 15 days post-spawn for walleye (McElman and Balon 1979), and 27 days post-spawn for yellow perch (Mansueti 1964). Diets in baseline simulations were based on our results from prey selection experiments, with both predators consuming zooplankton until they reached 20 mm. Thereafter, walleye consumed only fish, whereas yellow perch consumed equal proportions of fish, zooplankton, and benthic invertebrates. Caloric densities of prey items were estimated from Cummins and Wuycheck (1971). Predator lengths (mm) were converted to weights, and vice versa, with the length–weight equation developed for walleye and yellow perch in Lake Mendota (Madenjian and Carpenter 1991). We first ran initial simulations for both predators until they reached the average size at age-1, as reported by Mittelbach and Persson (1998; modified from Winemiller and Rose 1992), at the end of one growing season (80 g for walleye, and 5.8 g for yellow perch). Baseline simulations encompassed the entire growing season (April–November) for Lake Mendota, running 228 days for walleye and 213 days for yellow perch. The proportion of maximum consumption (p value) was standardized at 0.75 for the initial simulation and served as our baseline for comparison in subsequent simulations.

The effect of hatching date was examined by hatching yellow perch on the same day as walleye in the walleye baseline but holding all other model parameters constant. Walleye were similarly hatched on the same day as the yellow perch baseline hatching day while other parameters were held constant. To determine the tradeoffs in growth for a piscivorous diet versus an invertebrate-dominated diet for both predators, we modeled yellow perch growth assuming they switched to a 100% fish diet at 20 mm (the same as walleye baseline). Conversely, walleye growth was modeled assuming a mixed diet containing equal proportions of fish, zooplankton, and benthic invertebrates (the same as the yellow perch baseline). Finally, the effect of hatching date and diet in combination was examined by hatching yellow perch at the same time and with the same diet as walleye, and vice versa.

Results

Prey selection

When offered a high proportion of fish prey relative to benthic invertebrates and zooplankton, yellow perch exhibited neutral selection for fish across all sizes, except at 80 mm, where they showed weak positive selection (Fig. 1). Conversely, walleye always exhibited positive selection for fish prey. Walleye avoided benthic invertebrates at 20 and 40 mm, but exhibited neutral selection for them at 60 and 80 mm. Yellow perch positively selected benthic invertebrates at 60 mm, but showed neutral selection at 40 and 80 mm, and avoided them at 20 mm. Yellow perch exhibited neutral selection for zooplankton at 20 and 40 mm, whereas walleye always avoided zooplankton when fish prey were abundant. Overall, yellow perch did not positively select fish prey, despite the high proportion of fish, compared with alternate invertebrate prey. Yellow perch instead selected a mixed diet of fish and zooplankton at smaller sizes and fish and benthic invertebrates at larger sizes. Walleye positively selected fish across all sizes and showed neutral selection for benthic invertebrates at larger sizes.

When offered a prey assemblage of low fish density and high invertebrate densities, walleye positively selected fish at all sizes except 20 mm, and yellow perch did not positively select fish at any size (Fig. 1). Yellow perch positively selected benthic invertebrates at 40 mm, but exhibited neutral selection for chironomids at other sizes. Walleye neutrally selected benthic invertebrates at 20 and 60 mm, but avoided chironomids at 40 and 80 mm. Yellow perch positively selected zooplankton only at 20 mm, whereas walleye avoided zooplankton at all larger sizes (walleye neutrally selected zooplankton at 20 mm). Even when offered fish prey at lower densities relative to alternate prey, walleye maintained positive selection for fish whereas yellow perch exhibited negative to neutral selection for fish prey.

Growth

Growth rate (mm·mm^{-1}·day^{-1}) was highly variable and interacted significantly across three factors (predator, predator size, and prey type, $F_{[6,134]} = 3.41$, $P < 0.01$). Therefore, we compared the main effect of predator (yellow perch versus walleye) within levels of predator size and prey taxa. Walleye grew faster than yellow perch when feeding on fish prey at 20, 60, and 80 mm (t test$_{134}$ = 2.67–6.04, P = 0.05 – < 0.01; Fig. 2). However, yellow perch grew faster than walleye at 40 mm on fish (t test$_{134}$ = 3.14, P = 0.02). Walleye and yellow perch gained similar length while feeding on benthic invertebrate prey at all sizes except 80 mm, where walleye grew faster than yellow perch (t test$_{134}$ = 3.21, P = 0.01). Yellow perch grew faster on zooplankton prey than did walleye at 40 and 60 mm (t test$_{134}$ = 5.72 and 4.84, $P < 0.01$), but both predators had similar growth rates at 20 mm (t test$_{134}$ = 0.54, P = 0.59) and 80 mm (t test$_{134}$ = 0.7, P = 0.48) while consuming zooplankton.

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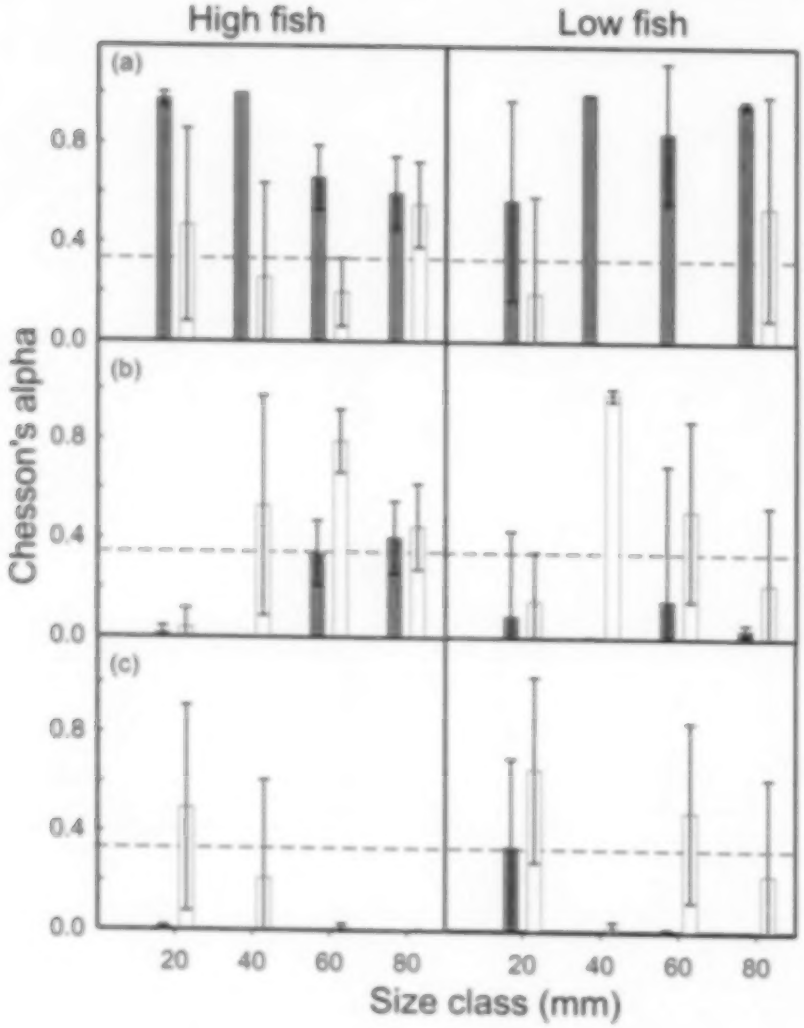
Fig. 1. Chesson's alpha (mean ± 95% confidence interval) for walleye (*Sander vitreus*; shaded bars) and yellow perch (*Perca flavescens*; open bars) across different size classes and prey types: (*a*) fish, (*b*) benthic invertebrates, and (*c*) zooplankton. Fish prey were offered at high (left panels) and low (right panels) densities relative to benthic invertebrates and zooplankton. Broken lines indicate random feeding. Values above, below, and near the line indicate positive selection, negative selection, and neutral selection, respectively.



Growth in weight ($g \cdot g^{-1} \cdot day^{-1}$) was also significantly affected by a three-factor interaction (predator, predator size, and prey type, $F_{[6,134]} = 2.36$, $P = 0.03$). The smallest size class (20 mm) of walleye gained more mass than the smallest yellow perch size class (20 mm) while feeding on fish prey (t test$_{134} = 4.43$, $P < 0.01$). Growth was similar for both predators across all larger sizes when feeding on fish prey (t test$_{134} = 0.62–2.75$, $P = 0.07–1$). Walleye and yellow perch gained mass at similar rates across all sizes while feeding on benthic invertebrate prey (t test$_{134} = 0.16–2.34$, $P = 0.18–1$). Yellow perch gained mass faster than walleye when feeding on zooplankton at 60 mm (t test$_{134} = 3.31$, $P = 0.01$). Overall, growth in weight was relatively similar for both predators across all sizes and prey types. However, allometric weight differences between the two predators (i.e., yellow perch tended to have greater mass at a given length than walleye) resulted in growth-in-weight comparisons being less robust.

Feeding behavior

Attack rates (number of strikes per minute) were higher for yellow perch than for walleye across all sizes and prey types ($F_{[1,201]} = 5.47$, $P = 0.02$). Attack rates were also significantly affected by a two-factor interaction (predator size and prey taxa, $F_{[6,201]} = 3.47$, $P < 0.01$). Within a size class, attack rates were highest for zooplankton, intermediate for benthic invertebrates, and lowest for fish (t test$_{201} = 2.36–9.71$, $P = 0.03 – <0.01$; Fig. 3), except at 80 mm, where at-

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Fig. 2. Relative growth rate (mm·mm^{-1}·day^{-1}) of juvenile walleye (*Sander vitreus*; shaded bars) and yellow perch (*Perca flavescens*; open bars) across different sizes while feeding on (*a*) larval fish, (*b*) benthic invertebrates, and (*c*) zooplankton. Values are means ± 95% confidence intervals. Significant differences denoted by asterisks were evaluated for pairwise comparisons of walleye and yellow perch at each size and prey type.

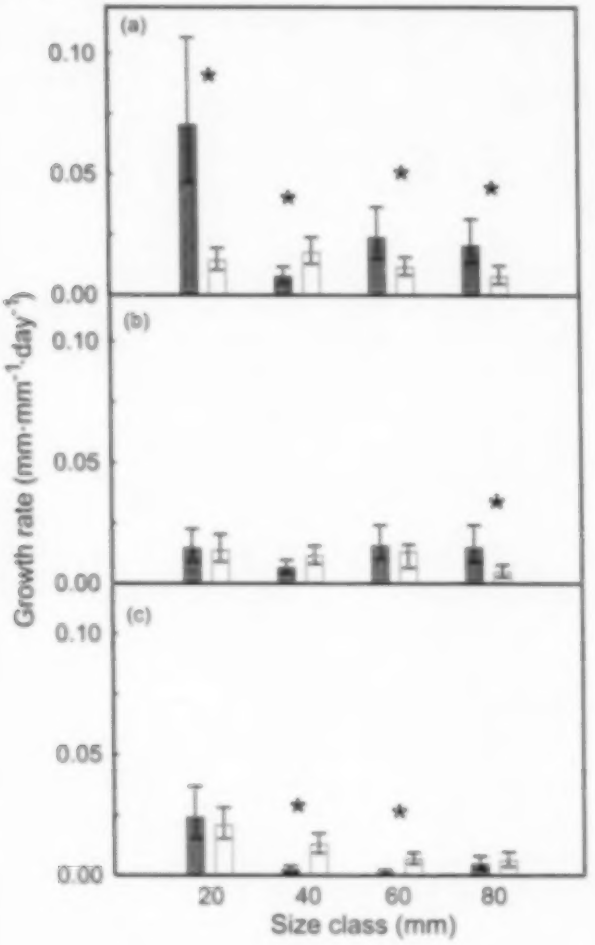
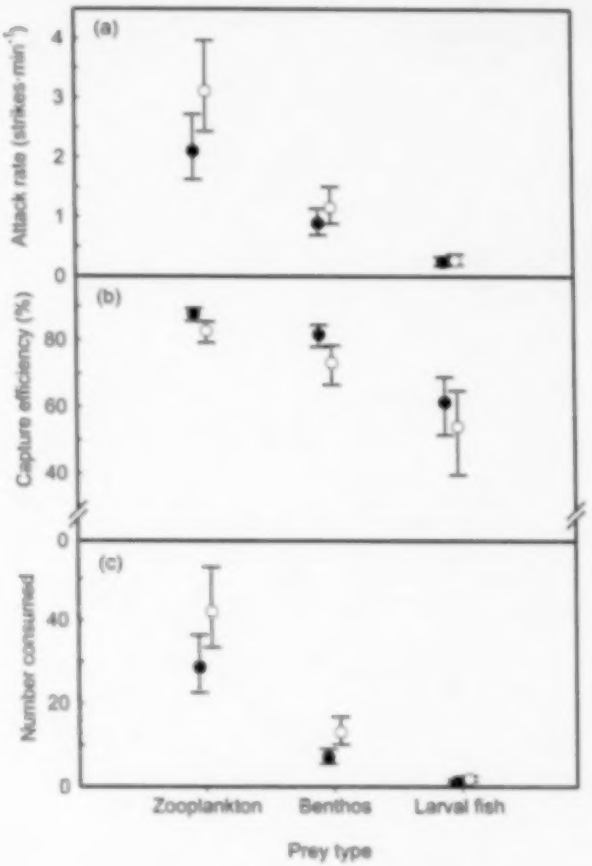


Fig. 3. Walleye (*Sander vitreus*; ●) and yellow perch (*Perca flavescens*; ○) foraging behavior on (*a*) fish, (*b*) benthic invertebrates, and (*c*) zooplankton. All behaviors were quantified during 15-min feeding trials. Values are means ± 95% confidence intervals.



tack rates were similar for benthic invertebrates and zooplankton (t test$_{201}$ = 1.45, P = 0.15). However, these relationships were similar for walleye and yellow perch, as there was no interaction between predator and either predator length ($F_{[3,201]}$ = 1.9, P = 0.13) or prey type ($F_{[3,201]}$ = 0.68, P = 0.51). Prey density also significantly affected attack rate, with both predators exhibiting higher attack rates at high prey densities (1.25 strikes·min^{-1}) compared with low prey densities (0.65 strikes·min^{-1}, t test$_{201}$ = 5.92, P < 0.01).

Capture efficiency (the number of strikes per capture) was significantly affected by predator and prey type. Walleye exhibited higher capture efficiency than yellow perch ($F_{[1,193]}$ = 13.67, P < 0.01). Capture efficiency also differed among prey types for both predators ($F_{[2,193]}$ = 50.43, P < 0.01). Capture efficiency was highest for zooplankton, intermediate for benthic invertebrates, and lowest for fish prey (Fig. 3). Capture efficiency was not influenced by predator length ($F_{[3,193]}$ = 1.66, P = 0.18) or prey density ($F_{[1,193]}$ = 2.48, P = 0.12). Thus, walleye captured prey more efficiently than yellow perch; capture efficiency was higher for both predators while consuming smaller prey items. These patterns were consistent across all predator sizes and both prey densities.

Unlike attack rate and capture efficiency, the number of prey items consumed differed little between yellow perch and walleye (Fig. 3). Because there was a significant three-factor interaction (predator, predator size, and prey type, $F_{[6,201]}$ = 2.81, P = 0.01), we again compared the main effect of predator within levels of predator size and prey type. At 20 mm, yellow perch consumed significantly more zooplankton than did walleye (t test$_{201}$ = 4.06, P = 0.02). For all other size classes and prey types, yellow perch and walleye consumed similar numbers of prey items (t test$_{201}$ = 0.33–2.66, P = 0.09–1). Given that the significant three-factor interaction was driven by only one size class and prey type,

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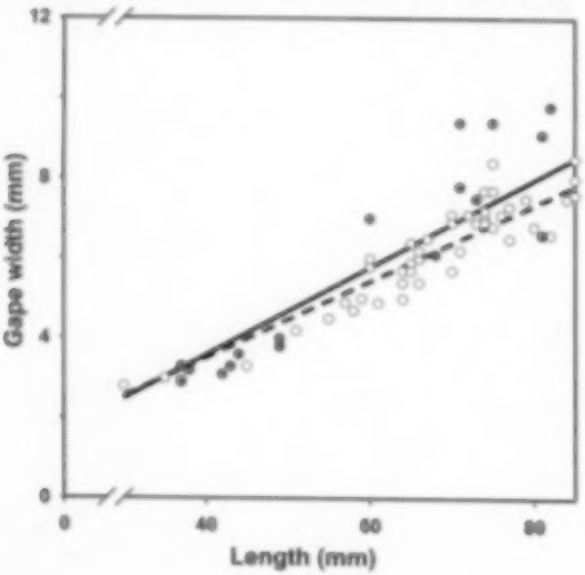
Table 1. Final weight, consumption, and growth efficiency for yellow perch (*Perca flavescens*) and walleye (*Sander vitreus*) from bioenergetic model simulations.

Predator	Model simulation	Final weight (g)	Total consumption (g)	Growth efficiency (%)
Yellow perch	Initial	6	80	8
	Baseline (p value = 0.75)	11	126	9
	Early hatch date	11	126	8
	Fish diet	39	245	16
	Early hatch date and fish diet	40	253	16
Walleye	Initial	80	432	19
	Baseline (p value = 0.75)	53	308	17
	Late hatch date	51	297	17
	Mixed diet	2	34	6
	Late hatch date and mixed diet	2	32	6

Note: Baseline p value (0.75; proportion of maximum consumption) resulted from initial simulations (average values of growth, diet, and temperature from our study and literature). For both predators, we also simulated switched hatching dates, diets, and both hatching dates and diets in combination.

Fig. 4. Gape width-to-length relationships of walleye (*Sander vitreus*; —●—) and yellow perch (*Perca flavescens*; - - ○ - -). Gape width was measured at the narrowest point in the mouth using calipers. Linear regression was used to predict gape width based on total length of predator and provided good fits for both predators ($r^2 = 0.87$, $P < 0.01$ for both walleye and yellow perch).



we conclude that walleye and yellow perch generally consumed similar numbers of each prey type. However, both yellow perch and walleye did consume more prey in the high-density prey treatments compared with the low-density treatments ($F_{(1,201)} = 28.78$, $P < 0.01$).

Morphology

Length predicted gape width for both walleye ($r^2 = 0.87$, $P < 0.01$) and yellow perch ($r^2 = 0.87$, $P < 0.01$; Fig. 4). The equations to estimate gape G based on predator length L were as follows: $G = -1 + 0.11L$ for walleye, and $G = -0.46 + 0.1L$ for yellow perch. Initially, gape widths were similar for both predators (ANCOVA (intercept) = 1.85, $P = 0.07$), but walleye gape widths increased at a faster rate than those of yellow perch (ANCOVA (slope) = 2.26, $P = 0.03$).

Bioenergetic model simulations

In initial simulations, the p value needed by walleye to reach its final weight was 0.79; for yellow perch, the p value was 0.65. With standardized p values (p value = 0.75), consumption and growth efficiency were slightly higher for yellow perch and reduced for walleye compared with initial simulations (Table 1). Hatching date alone had little effect on growth for both predators. When hatching dates were switched, but diets remained the same, size at the end of the growing season, consumption, and growth efficiency either remained the same (yellow perch) or decreased slightly (walleye) from baseline simulations with standardized p values (Fig. 5). In contrast, the proportion of fish in the diet strongly influenced growth for both predators; however, neither predator achieved growth comparable to the baseline of the other. When yellow perch were allowed to switch to a piscivorous diet at 20 mm, their length at the end of the growing season remained 24% lower than for the walleye baseline simulation (Fig. 5). Similarly, when walleye were forced to maintain a mixed diet with equal proportions of fish, benthic invertebrates, and zooplankton, growth was much slower and their size at the end of the growing season was 82% lower than that of yellow perch. When both hatching date and diet were changed, growth and consumption of both predators remained similar to simulations with switched diets only (Table 1; Fig. 5). Overall, walleye realized faster growth rates than yellow perch on a piscivorous diet, but grew much slower than yellow perch on a mixed diet. Conversely, yellow perch did not grow as fast as walleye on a piscivorous diet, but they were able to grow faster than walleye on a mixed diet.

Discussion

As predicted, walleye, as specialist piscivores, strongly selected fish prey over alternate prey, whereas yellow perch, as generalist predators, displayed negative to neutral selection of fish and favored alternate prey types. These patterns held

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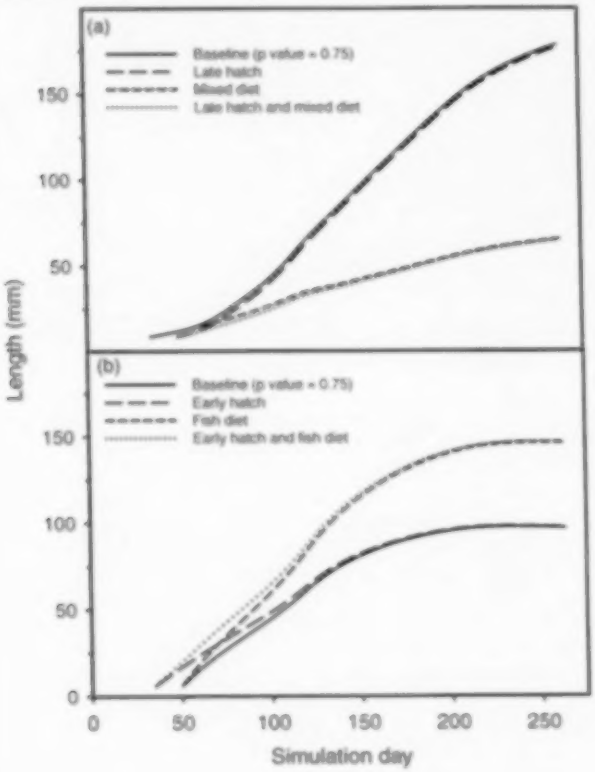
Fig. 5. Lengths (mm) of age-0 (*a*) walleye (*Sander vitreus*) and (*b*) yellow perch (*Perca flavescens*) over one growing season (April–November) from bioenergetic model simulations. Baseline p value (0.75; proportion of maximum consumption) resulted from initial simulations (average values of growth, diet, and temperature from our study and literature). For both predators, we also simulated switched hatching dates, diets, and both hatching dates and diets in combination.



even when fish prey were offered in lower proportion relative to alternate prey types. Selection patterns were supported by differences in growth of juvenile yellow perch and walleye. Walleye grew faster than yellow perch while feeding on fish prey across most sizes. Interestingly, regardless of walleye size, walleye also grew faster on fish prey compared with other prey types, whereas yellow perch growth varied little across all prey types. We believe that an important reason for this pattern of growth and selection is that walleye actively selected fish prey, likely based on optimal foraging, whereas yellow perch passively selected all prey based on availability and foraging efficiency.

Growth and prey selection from our experiments generally support patterns observed in the field. Within the range of sizes we examined, field data for juvenile walleye showed positive selection for, and strong growth while consuming, fish prey (Mathias and Li 1982). Piscivory is also common during the juvenile period for hybrid saugeye (*Sander canadensis* × *S. vitreus*; Donovan et al. 1997), and in closely related pikeperch (*Sander lucioperca*; Buijse and Houthuijzen 1992). These predators realize a substantial growth increase after switching to piscivory. Field patterns for growth and prey selection by yellow perch also support our results. Although fish prey has been observed in diets of yellow perch as small as 65 mm, juvenile yellow perch generally consume invertebrates and realize high growth when these prey are abundant (Mills et al. 1989; Fullhart et al. 2002). Thus, in the field, walleye are piscivorous across the range of juvenile sizes that we examined, whereas yellow perch are generalist predators, choosing a diet that may contain fish but is dominated by invertebrates.

Foraging behavior supported, but did not fully explain, the observed differences in prey selection and growth patterns between walleye and yellow perch. Capture efficiency was higher for walleye than yellow perch while feeding on fish prey, suggesting that walleye must expend less energy than yellow perch in capturing fish prey (Einfalt and Wahl 1997). However, it is not clear if the relatively small difference in overall foraging efficiency on fish prey between walleye and yellow perch (e.g., capture efficiency: 61% versus 54%) is large enough to account for the very large differences we observed in prey selection and growth. In a study of selective feeding and growth for esocids, capture efficiencies were greater than threefold more for fusiform prey (>60%) over bluegill (<20%) and these differences conferred higher growth (Wahl and Stein 1988). In contrast with capture efficiency, the overall attack rate of walleye was lower than that of yellow perch. However, because walleye captured prey more efficiently, both predators consumed similar numbers of prey items. Yellow perch attacked prey more frequently to overcome lower capture efficiency. As a consequence, yellow perch likely expended more energy to obtain the same caloric intake as walleye. Given their higher overall foraging efficiency, we expected walleye to outgrow yellow perch for all size classes and prey types (particularly fish prey). However, yellow perch grew faster than walleye while feeding on fish prey at 40 mm, and on zooplankton at 40 and 60 mm. Our results suggest that walleye are fish specialists that actively select their prey. As such, growth suffers accordingly when fish prey are not available, even though walleye forage more efficiently on each prey type compared with perch.

Mouth morphology also differed between yellow perch and walleye, particularly for larger sizes. Predator morphology (particularly gape width) is an important factor regulating piscivory (Olson 1996; Einfalt and Wahl 1997) because larger gapes allow piscivores to capture and handle fish prey more effectively, and small differences could affect foraging success. We held fish prey size constant across size classes of predator (i.e., 20%–25% total length) to provide a constant and optimal predator:prey size ratio. We also ensured that the fish prey used in our experiments were within the limits of gape width for both predators. Because walleye are piscivorous at smaller sizes than yellow perch, we predicted that walleye would have larger gapes across all sizes. In fact, gape widths were similar across smaller sizes of both predators, but walleye gape increased faster than that of yellow perch, resulting in larger gapes for walleye at larger sizes. This may help explain why larger walleye grew better while consuming fish and selected this prey type more strongly than did yellow perch. However, growth and selection differed between predators when gape widths did not differ at smaller sizes.

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Although increased gape might explain why larger walleye select for fish prey compared with yellow perch, other factors regulate the differences in selection at smaller sizes. The presence of canine teeth is one morphological attribute that may contribute to the different selection patterns and foraging efficiency observed for walleye compared with yellow perch. Canine teeth may allow walleye to grasp and hold onto prey items more effectively than yellow perch (Craig 1987), allowing them to feed more efficiently on prey that can escape capture. Overall, predator morphology was also an important determinant of prey selection and growth, but did not fully explain the differences in dietary ontogeny between walleye and yellow perch.

The difference in timing of piscivory between walleye and yellow perch is likely a result of an interaction between foraging success and gape width. Although the differences in foraging efficiency and gape width between walleye and yellow perch were relatively small, they always favored walleye to become piscivorous earlier than yellow perch. These conclusions differ slightly from Mittelbach and Persson (1998), who hypothesized that most of the variation in the ontogeny of piscivory was explained by morphological differences among predators. Predators in our study had similar gape widths at smaller sizes (20 and 40 mm), but walleye still foraged more efficiently on fish prey compared with yellow perch. Mittelbach and Persson (1998) speculated that early switching piscivores would also have a higher overall foraging efficiency than fish species switching at larger sizes.

Walleye were slightly more efficient foragers across all sizes and prey types, but the magnitude of differences were much smaller than we predicted. Based solely on foraging efficiency, we would have expected walleye to grow faster than yellow perch on all prey types. Because walleye grew faster only with fish prey, we conclude that walleye were actively selecting fish prey based on optimal foraging considerations, which explains why walleye select fish so much earlier than yellow perch, which passively select their prey. Furthermore, fish prey, which have a higher caloric density than invertebrates (3698 $J \cdot g^{-1}$ for fish prey versus 2600 $J \cdot g^{-1}$ for zooplankton and 1763 $J \cdot g^{-1}$ for benthic invertebrates; Cummins and Wuycheck (1971)), reinforce their active selection by the specialist piscivore. Conversely, yellow perch passively select prey based on foraging efficiency and prey availability alone. Thus, it is likely that both predator morphology and foraging efficiency influence the ontogeny of piscivory, but consideration of selection mode, in the context of optimal foraging, further explains the observed differences between walleye and yellow perch.

The importance of life history variation in determining piscivory was also suggested by Mittelbach and Persson (1998). Although they could not detect a statistical relationship, they speculated that the timing and size at hatching contributed to differences in dietary ontogeny. We explored the role of hatching dates and the ability to consume only fish using bioenergetic simulations. The shift to piscivory is facilitated by the appearance of prey fish availability. Piscivores large enough to consume fish prey as they become available (synchronous) switch early to piscivory and maintain fast growth rates, whereas piscivores poorly matched with the appearance of fish prey are unable to switch to piscivory and maintain slower growth (Persson and Brönmark 2002). Walleye hatch earlier than yellow perch and at larger sizes; as such, walleye likely gain a size advantage over other age-0 fishes. Conversely, later hatched yellow perch may not achieve a size advantage over fish prey. These differences may enable walleye to grow faster and switch to piscivory earlier than yellow perch simply because they have more fish prey available within gape limits. Our bioenergetic simulations showed that hatching 15 days earlier (yellow perch with walleye hatching date) or later (walleye with yellow perch hatching date) had little effect on growth when diets remained the same. However, if hatching early allowed either predator to switch to piscivory, they realized increased growth compared with maintaining a mixed diet. Thus, hatching time of predators relative to the appearance of fish prey is likely important in facilitating the switch to piscivory, but this mechanism should be further tested in a field setting.

Our bioenergetic simulations were designed to be broadly representative of conditions across the range of both predators. As such, values we used as model parameters were often used or modified from values reported in the literature, but these can be variable. For example, we used 27 days as the duration from hatching to exogenous feeding in our simulations for yellow perch (Mansueti 1964), but values as short as 15 days have also been reported (Scott and Crossman 1973). Both hatching date and duration to exogenous feeding are likely affected by temperature. Even with the incorporation of this variation, our simulations would still indicate that timing of hatching and switching to exogenous feeding had little effect on growth for both predators when diets were held constant. Switching to a piscivorous diet, however, had a very large effect on growth for both predators. We based model diet shifts on results from our prey selection experiments in which walleye positively selected fish across all sizes and yellow perch did not show distinct selection patterns. However, we recognize that field studies have documented variation in diet shifts. For example, walleye have been shown to switch to piscivory at larger sizes than we used for our simulations (i.e., 35 mm in Mathias and Li 1982, compared with 20 mm in this study). Similarly, previous studies of yellow perch food habits show they can have a varied diet, but it is composed mostly of invertebrates for the first several years of life (Fullhart et al. 2002). Despite the potential variation in food habits for these two predators, the importance of fish as prey remained unchanged across a range of prey availabilities in our simulations.

Our growth simulations address the following question: Do specialist piscivores take a higher risk in terms of growth by specializing on fish prey? Specifically, when fish prey are not available to specialist predators, will they experience lower growth than a generalist predator? Because walleye actively seek fish prey based on optimal foraging, when fish prey are unavailable, growth is much slower when walleye maintain and invertebrate diet. Conversely, yellow perch passively select prey based on foraging efficiency and maintain similar growth on invertebrate and (or) piscivorous diets as long as either prey type is efficiently consumed. Mittelbach and Persson (1998) thought that, as specialist piscivores grew, foraging efficiency on non-fish prey would rapidly decline, whereas generalist predators would experience slower

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decreases in foraging efficiency on non-fish prey. Our simulations showed that walleye forced to maintain a mixed diet exhibited growth rates much lower than that of yellow perch with a similar diet. We are unsure why growth of walleye was lower than that of yellow perch on a non-fish diet (and conversely, why walleyes grow faster than perch on a piscivorous diet), but other feeding behaviors, such as reactive distance and pursuit, as well as metabolic allometry, would better address optimal foraging and should be considered in future studies. Walleye are specialist piscivores that actively select fish prey; when they switched to piscivory at a small size, they realized very fast growth, but growth was much reduced when walleye could not switch to piscivory. Yellow perch are generalist predators; they did not grow as fast as walleye with a fish diet, but grow faster than walleye with an invertebrate diet.

Walleye, as specialist piscivores, switch to piscivory earlier than yellow perch, and the increase in growth associated with a fish diet may allow them to maintain a size advantage over fish prey throughout the growing season. The early hatching of walleye may help facilitate the switch to piscivory because other fishes hatch later than walleye, but has little direct influence on growth. Conversely, if yellow perch do not switch to piscivory early they realize slower growth, precluding them from switching to piscivory until age 1+. This delayed switch to piscivory may be further exacerbated by the later hatching of yellow perch because other prey fish hatch before or during the same time as yellow perch. As such, differences in feeding ontogeny between walleye and yellow perch may result simply because yellow perch never realize a size advantage over the majority of fish prey available. Life history variation is an important factor affecting the ontogeny of piscivory; however, additional research to understand the mechanisms facilitating the switch to piscivory during early life history, specifically the interactive effects of hatch date and prey fish abundance, is needed.

Our results indicate that the onset of piscivory is driven by a combination of several factors. We investigated three potential factors and found that each supported, but did not fully explain, the patterns of growth and prey selection between walleye and yellow perch. The specialist piscivore (walleye) foraged more efficiently on fish prey, had a mouth gape that increased rapidly, and hatched earlier than the generalist predator (yellow perch). As such, walleye grew faster on fish prey than yellow perch, and strongly selected for fish, whereas yellow perch selected all three prey types. Our results suggest that walleye are actively selecting fish prey based on optimal foraging, whereas yellow perch passively select fish prey based on encounter rates and foraging efficiency. These characteristics in combination help to explain why some fish species switch to piscivory early, whereas others do not.

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Persistent organic pollutants in river food webs: influence of trophic position and degree of heterotrophy

Olof Berglund, Per Nyström, and Per Larsson

Abstract: We investigated how the degree of autotrophy/heterotrophy and organism trophic position influenced the bioaccumulation of polychlorinated biphenyls (PCBs) in 10 benthic river food webs consisting of terrestrial detritus, periphyton, invertebrates, and age-0 brown trout (*Salmo trutta*) in southern Sweden. Concentrations of PCBs increased with trophic position, estimated from $\delta^{15}N$ and $\delta^{13}C$, on a dry weight basis ($ng \cdot g^{-1}$ dry weight) but not on a lipid weight basis ($ng \cdot g^{-1}$ lipid). PCB biomagnification factors between the first and second trophic levels (invertebrates/periphyton and invertebrates/detritus) ranged between 0.3 and 2.3 and between the second and third levels (trout/invertebrates) between 0.3 and 2.0 on a lipid weight basis. The mean proportion of carbon ultimately derived from terrestrial sources, α, was 0.82 ± 0.19 for invertebrates and 0.67 ± 0.28 for trout. Contrary to our hypothesis, PCB concentrations in trout were positively related to α (r^2 = 0.58–0.77, $p < 0.05$). As α and the periphyton density ($g\ C \cdot m^{-2}$) in the rivers was positively related (r^2 = 0.88, $p < 0.01$), we propose that this relationship was due to an increased retention and exposure of PCBs to trout in rivers with low grazing pressure and high periphyton density.

Résumé : Nous avons déterminé dans quelle mesure le degré d'autotrophie/hétérotrophie et la position trophique des organismes influencent la bioconcentration des biphényls polychlorés (BPC) dans 10 réseaux alimentaires benthiques de rivière comprenant le détritus terrestre, le périphyton, les invertébrés et les truites brunes (*Salmo trutta*) d'âge 0 dans le sud de la Suède. Les concentrations de BPC augmentent en fonction de la position trophique lorsqu'elles sont estimées à partir de $\delta^{15}N$ et de $\delta^{13}C$ en masse sèche ($ng \cdot g^{-1}$ de masse sèche), mais pas en masse lipidique ($ng \cdot g^{-1}$ de lipides). Les facteurs de bioamplification (BMF) entre les premier et second niveaux trophiques (invertébrés/périphyton et invertébrés/détritus) varient de 0,3 à 2,3 et entre les second et troisième niveaux (truite/invertébrés) de 0,3 à 2,0 sur la base de la masse lipidique. La proportion moyenne de carbone dérivée en fin de compte des sources terrestres, α, est de 0,82 ± 0,19 chez les invertébrés et de 0,67 ± 0,28 chez les truites. Contrairement à notre hypothèse, les concentrations de BPC chez la truite sont en corrélation positive avec α (r^2 = 0,58–0,77, $p < 0,05$). Comme α est relié directement (r^2 = 0,88, $p < 0,01$) à la densité du périphyton ($g\ C \cdot m^{-2}$) dans les rivières, nous croyons que cette relation est due à une rétention accrue et une exposition plus grande des BPC chez les truites dans les rivières à faible pression de broutage et à forte densité de périphyton.

[Traduit par la Rédaction]

Introduction

The contamination of waters with persistent organic pollutants (POPs) has occurred over the last four decades and is still a major environmental issue in aquatic environments. Although levels of certain compounds appear to have decreased after prohibition in many industrialized countries in the last two decades, the decrease today seems to have leveled off and high concentrations, especially in aquatic predators, are still of concern (Bignert et al. 1998; Nyman et al. 2002). Also, new POPs have replaced old ones and these new compounds are now found in increasing levels in aquatic environments (Rayne et al. 2003). Another environmental concern in aquatic environments is the eutrophication caused by excessive load of nutrients from anthropogenic activities. The main effect of eutrophication on aquatic ecosystems is an increase of primary production. In rivers, it has been demonstrated that the addition of phosphorus shifts the system from heterotrophy, where detritus of terrestrial origin is the dominating carbon source, to autotrophy, where aquatic primary producers are the dominating carbon source (Peterson et al. 1985). Earlier, we have shown that POPs in river biota increase with increasing trophic status of the river (Berglund et al. 1997). We suggested that a shift from terrestrial carbon sources to aquatic might be partly responsible for the increase of POP concentrations, possibly owing to initial differences in POP concentrations in the primary producers. These differences at the first trophic level may persist in higher trophic levels of the aquatic food web owing to food chain transfer of POPs. POPs are often found in high concentrations in the top predators of aquatic food chains at

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concentrations above those predicted from equilibrium partitioning between the water and the organism (Fisk et al. 2001). This phenomenon has been attributed to the influence of food and the process of biomagnification where piscivorous fish reach higher concentrations than the prey owing to gastrointestinal (GI) magnification (Connolly and Pedersen 1988; Gobas et al. 1999). If this mechanism applies to all trophic transfers between consumers and food in the food web, it could lead to food chain bioaccumulation, an increase in concentration with each step in the food chain. The studies on biomagnification and food chain bioaccumulation of POPs in aquatic environments have predominantly been focused on predatory pelagic fish or mammals in lakes and marine environments. Benthic food webs including the base and lower trophic levels, and in particular those in lotic environments, have thus far been largely neglected, with a few exceptions (Galassi et al. 1994; Hill and Napolitano 1997; Zaranko et al. 1997).

The ratio of stable isotopes of nitrogen ($\delta^{15}N$) can be used to estimate trophic position because the $\delta^{15}N$ of a consumer is typically enriched by 3–4‰ relative to its diet (Peterson and Fry 1987). Therefore, estimates of $\delta^{15}N$ of the different components in food webs are often used to investigate the effect of trophic position and biomagnification on POP concentrations in food webs. The ratio of carbon isotopes ($\delta^{13}C$), however, seems to change little in the transfer between trophic levels (Peterson and Fry 1987; France and Peters 1997) and may therefore be used to evaluate the sources of origin of carbon in an organism when the isotopic signatures of the sources are different. Terrestrial and aquatic primary producers have been found to differ in $\delta^{13}C$ signature (Peterson and Fry 1987), and therefore, $\delta^{13}C$ signatures of the components in aquatic food webs may be used to estimate the influence of heterotrophy where carbon is derived from terrestrial (allochthonous) sources versus autotrophy where the carbon is derived from aquatic (autochthonous) sources in the system. There is considerable variation, however, among ecosystems in the $\delta^{15}N$ and $\delta^{13}C$ of the base of the food web from which organisms draw their nitrogen and carbon, and also, there may be more than one base food source (Nyström et al. 2003). By constructing mixing models and combining $\delta^{15}N$ and $\delta^{13}C$ signatures of the different base food sources and consumers, it may be possible to calculate the influence of different food sources and trophic positions of consumers that are comparable across different ecosystems (Post 2002).

Here, we wanted to investigate the influence of the degree of heterotrophy/autotrophy on POP concentrations in the river food chains by testing the hypothesis that POP levels are higher in aquatic autotrophs than in terrestrial detritus, and if so, these differences are preserved in the autotrophic food chain. The degree of heterotrophy/autotrophy, α, was determined from the $\delta^{13}C$ ratios in detritus of terrestrial origin, periphyton, invertebrates, and age-0 brown trout (*Salmo trutta*). Further, we wanted to investigate the food chain bioaccumulation of POPs in rivers to test the hypothesis that POP concentrations in river organisms increase with increasing trophic position of the organism and if the concentrations in the food chain could be explained by equilibrium partitioning, steady-state biomagnification, or non-steady-state bioaccumulation. We investigated polychlorinated biphenyl (PCB) concentrations in the food chains of 10 streams and rivers in southern Sweden. The compartments of the food chains in each river were detritus of terrestrial origin, periphyton, invertebrates, and age-0 trout. The trophic position of the compartments were assigned as traditional discrete levels (1, 2, and 3), on a continuous scale from $\delta^{15}N$ measurements, and from combining $\delta^{13}C$ and $\delta^{15}N$ values in the compartments and in the different food sources in a mixing model as described by Post (2002).

Materials and methods

Stream characteristics and sample collection

The sampling was conducted from July to October 1998. Ten different rivers in southern Sweden (13°E, 56°N) were selected for the experiment. The rivers were chosen on a trophic gradient determined by the total phosphorus concentrations in the water. The rivers are situated in a defined geographical area that receives similar amounts of organochlorines via atmospheric deposition with no known point sources (Berglund et al. 1997; Backe et al. 2000). The river stretches where the sampling was performed were similar in length (25 m) and riffle parts of the rivers were always included. River characteristics are presented in Table 1. Periphyton was sampled on ceramic tiles. Twenty 20 cm × 20 cm tiles were placed in each of the 10 rivers. The tiles were left for colonization for 60 days from July to October. At the end of the colonization period, the tiles were scraped with a brush into one precleaned container giving one pooled sample for each river. The periphyton density was calculated as milligrams of organic carbon per square metre. Organic carbon content was determined for terrestrial primary producers, periphyton, and detritus with an LECO CR-12 carbon analyzer (Merry and Spounser 1988). One site was not included in the analysis of periphyton density, as the tiles were destroyed during the experiment. At this site, periphyton for stable isotope analysis was sampled by scraping stones from the river. After periphyton sampling, two or three individuals of age-0 trout were sampled with electrofishing, detritus of terrestrial origin (one pooled sample consisting mainly of leaves at varying stages of decomposition) was collected from the river, and terrestrial primary producers (one pooled sample consisting of leaves from alder (*Alnus* spp.), European beech (*Fagus sylvatica*), and sage (*Salix* spp.) and Vällingebäcken and Saxån grasses and leaves) were collected immediately adjacent to the river. Aquatic invertebrates were sampled by thoroughly kick-sampling the river stretches. All invertebrates from the kick-sampling were collected to represent potential food for age-0 trout and to give a crude representation of the invertebrate community in the rivers. The invertebrates were identified to group and then pooled after freeze-drying for PCB and stable isotope analysis. The invertebrate samples were in all rivers but one, Vällingebäcken, dominated by the shredder *Gammarus pulex* (70–100% of total dry weight) with varying amounts of snail (grazers) (>10%), leaches (invertebrate predators) (>15%), and mayflies (grazers) (>5%). The invertebrate sample from Vällingebäcken was composed of leaches (~25%), snails (~70%), and mayflies (~5%). *Gammarus pulex* and mayflies are important food sources for trout, while snails and leaches are part of the trout diet

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Table 1. Watershed area, land use in the watershed, morphological data, mean light penetration (±1 SD), mean total phosphorus concentrations (±1 SD), periphyton density, and mean age-0 brown trout (*Salmo trutta*) length (±1 SD) for the studied rivers.

Site	Watershed area (km^2)	Agriculture (%)	Rural (%)	Forest (%)	Mean width (m)	Mean depth (cm)	Mean current ($cm \cdot s^{-1}$)	Light penetration (%)	Total phosphorus ($\mu g \cdot L^{-1}$)	Periphyton density (mg $C \cdot m^{-2}$)	Trout length (mm)
Rönne å	15	5	5	90	2.5	10.3	6.3	19±6	37±4	6.2	70±7
Segeå	30	45	10	45	2.2	4.6	9.0	72±10	50±4	nm	99±2
Vallingebäcken	17	60	40	0	1.4	7.4	15.8	98±3	71±26	199.4	103±3
Hobybäcken	26	95	5	0	1.3	10.9	26.5	26±5	78±19	127.9	71±2
Höje å	131	25	5	70	4.8	25.9	5.9	34±17	86±19	10.0	113±8
Bråån	150	80	10	10	5.2	7.6	27.2	38±10	89±6	32.2	120±1
Saxån	30	90	10	0	3.4	19.0	8.2	90±2	100±50	21.3	76±4
Dybäcksån	42	90	5	5	2.3	7.1	4.1	24±31	101±32	70.0	66±2
Vallkärrabäcken	10	60	40	0	2.3	11.4	5.4	37±8	143±10	16.8	97±7
Mollebäcken	25	40	10	50	4.4	13.3	10.4	16±4	156±51	30.4	65±5

Note: nm, not measured.

but are not considered as important (e.g., Dahl 1998). All samples were frozen and then freeze-dried before analysis. Water samples for total phosphorus analysis were taken three different times during the periphyton colonization (beginning, middle, and end). Total phosphorus concentrations were analyzed with standard methods (digestion with peroxodisulphate) at the Department of Ecology, Lund University.

Sample preparation and analysis

The methods for sample preparation and analysis are described in detail elsewhere (Bremle et al. 1995). Briefly, PCBs in whole trout, invertebrates, periphyton, detritus, and terrestrial primary producers were extracted in a modified Soxhlet apparatus with a mixture of acetone and *n*-hexane. The lipid content of trout, invertebrates, periphyton, detritus, and terrestrial primary producers was determined gravimetrically. Octachloronaphthalene and pentachlorobenzene were used as internal standard and chromatographic standard, respectively. Concentrated extracts were cleaned and fractionated on acid–base double-layer silicagel columns. Samples were analyzed for PCBs by capillary gas chromatography with an electron capture detector (Varian 3400 cx) with a split/splitless injector, 25-m DB5 quartz capillary column (inner diameter 0.25 mm). PCB components were identified according to Mullin et al. (1984) and Schulz et al. (1989). A total of 65 peaks of PCB congeners were identified and quantified in the different samples. Domain 19 (IUPAC No. 52), D32 (No. 66/95), D38 (No. 90/101), D54 (No. 132/153/105), D58 (No. 160/138/158), D72 (No. 180), and D84 (No. 194) were used for further statistical analysis, as they were present in 100% of the samples in high concentrations and represent a wide range in lipophilicity (log octanol–water partitioning coefficient (log K_{OW}) 5.8–7.8) (Hawker and Connell 1988) and degree of chlorination (tetra- to octa-chlorinated).

Quality control

The analytical performance was regularly controlled with pesticide standards and PCB standards (Aroclor 1242 and Clophen A60). Extraction efficiency of the internal standard octachloronaphthalene was 113 ± 8%. Samples were not corrected for recovery. A procedural blank was run in parallel with every batch of eight samples. All blank concentrations of the seven PCBs used for statistical analysis were <6% of the levels found in the samples and no blank correction was made.

Stable isotope measurements

Carbon and nitrogen isotope ratios were analyzed in lipid-extracted samples of detritus, periphyton, invertebrates, and age-0 trout. The stable isotopes were analyzed on a continuous-flow IRMS (20–20 stable isotope analyser) (Europa Scientific Ltd., Crewe, UK) interfaced with an elemental analyzer unit (ANCA-NT system, solid–liquid preparation module) (Europa Scientific Ltd.) at the Department of Forest Ecology, Swedish University of Agricultural Sciences, Umeå, Sweden. A detailed description of the isotope analysis is given in Ohlsson and Wallmark (1999). Briefly, 1–5 mg of a finely ground sample was placed in tinfoil containers and masses were determined. The samples were then

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combusted completely in an oxidizing column (chromium oxide, copper oxide, and silver wool) and into which a pulse of oxygen was added concurrent with sample introduction. The carbon and nitrogen content of the sample was quantitatively converted into CO_2 and N_2, respectively. The CO_2 and N_2 formed were separated using a gas chromatography column and transferred with helium carrier gas through a thermal conductivity detector and then to the IRMS inlet. The notation is the relative difference in isotopic ratio between the samples and known standards (e.g., Peterson and Fry 1987; Ohlsson and Wallmark 1999).

To calculate the degree of heterotrophy, α, we used the equation from Post (2002):

$$(1) \qquad \alpha = (\delta^{13}C_c - \delta^{13}C_p)/(\delta^{13}C_d - \delta^{13}C_p)$$

where $\delta^{13}C_c$ is the isotopic signature of the consumer, $\delta^{13}C_p$ is the signature of periphyton, and $\delta^{13}C_d$ is the signature of detritus of terrestrial origin. When all carbon in the consumer is derived from terrestrial sources, $\alpha = 1$, and when all carbon is derived from aquatic sources, $\alpha = 0$.

To calculate a trophic position for a consumer comparable across all systems, we removed the effect of the between-site variability in the isotopic signatures at the base of the food webs and assumed a two-source food web with detritus of terrestrial origin and periphyton as the bases of the two food webs. The trophic position was calculated using the equation from Post (2002):

$$(2) \qquad \text{Trophic position} = \lambda + [\delta^{15}N_c - [\delta^{15}N_d\,\alpha + \delta^{15}N_p\,(1-\alpha)]]/\Delta_n$$

where λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ (e.g., $\lambda = 1$ for the primary producers used in this study) and Δ_n is the enrichment in $\delta^{15}N$ per trophic level (set to 3.4‰ in this study). The α is the proportion of nitrogen in the consumer ultimately derived from detritus of terrestrial origin and assuming that the movement of nitrogen and carbon through the food web is similar, identical to α from eq. 1.

Statistical analysis

All concentration and periphyton biomass data were $\log_{10}$-normalized before statistical analysis to obtain a normal distribution (Z scores, <5% of the data points > 2 or < −2). Biomagnification factors ($BMF_{consumer/food}$) for the different PCBs were calculated by dividing the dry weight (nanograms per gram dry weight) or lipid weight (nanograms per gram lipid) PCB concentration in the consumer with the dry weight or lipid weight concentration in the putative food. When testing $BMF_{invert/fish}$, we used means for each site to avoid pseudoreplication. Simple regression analyses tested the relationships between lipid content and PCB concentrations and the relationships between PCB concentrations or BMFs of the invertebrates or age-0 trout and trophic position or degree of heterotrophy (α). One-way ANOVAs with Tukey–Kramer post hoc tests ($\alpha = 0.05$) tested differences between dependent variables (lipid content, PCB concentrations, and BMFs) with the different compartments (terrestrial primary producers, detritus, periphyton, invertebrates, and age-0 trout) as the independent variable. ANCOVAs tested the significance of lipid content as covariate on PCB concentrations (nanograms per gram dry weight) with food web compartments as factors. One-sample t tests with hypothesized difference = 0 tested differences in log BMFs from zero. For repeated t tests, we used sequential Bonferroni tests by the Dunn–Šidák method to calculate critical α' values (Sokal and Rohlf 1995). All tests were performed using the StatView® package (SAS Institute Inc., Cary, North Carolina).

Results

Carbon source

The mean proportion of carbon ultimately derived from terrestrial sources, α, was 0.82 ± 0.19 for invertebrates and 0.67 ± 0.28 for trout. At several sites (five for invertebrates and three for trout), the estimated α value was >>1, i.e., the mixing model could not be used owing to the fact that the isotope ratios of periphyton and terrestrial detritus were overlapping or fell outside the limits of the invertebrate or trout isotope signatures (Nyström et al. 2003). These sites were excluded from statistical analyses involving α.

On a dry weight basis, higher K_{OW} PCB concentrations in trout were positively related to α, and all PCB concentrations were positively related to α on a lipid weight basis (Table 2). However, α was positively correlated with the periphyton density in the rivers (Fig. 1), which has earlier been shown to predict the organochlorine uptake in rivers (Berglund 2003). We removed the effect of periphyton density by performing simple regression analysis between periphyton density and PCB concentrations (simple regressions, $r^2 = 0.40$–0.58, df = 8, $p = 0.02$ – 0.07) and performed new regression analyses on α and the residuals from the former regressions. When the effect of periphyton density was removed, no relationships between α and PCB concentrations were found (simple regressions, df = 6, $p > 0.05$).

PCB concentrations did not differ greatly between the allochthonous (detritus and riparian vegetation) and autochthonous (periphyton) carbon sources. Generally, concentrations increased from terrestrial riparian vegetation ≤ detritus < periphyton, but differences were only statistically significant when expressed on a lipid or organic carbon basis (ANOVA, df = 2,24, Tukey–Kramer post hoc tests, $p < 0.05$) (Table 3). Mean terrestrial primary producer/periphyton concentration ratios ranged from 0.4 to 1.6 on a dry weight basis, from 0.1 to 0.4 on a lipid weight basis, and from 0.1 to 0.4 on an organic carbon basis, and mean detritus/periphyton ratios ranged from 0.7 to 1.8 on a dry weight basis, from 0.6 to 1.0 on a lipid weight basis, and from 0.3 to 0.6 on an organic carbon weight basis, with decreasing ratios with increasing K_{OW} of the compounds.

PCB concentrations and trophic position

Dry weight normalized concentrations of the seven PCB congeners were related to the lipid content of the samples from all the compartments in the food web (simple regressions, $r^2 = 0.26$–0.68, df = 57, $p < 0.05$) (Fig. 2). The degree of explanation and regression coefficients showed a bell-shaped relationship with the log K_{OW} of the compounds. However, within each compartment of the food web, we found no significant relationships between PCB concentrations and lipid content (the compartment × lipid content interaction terms and the effect of lipid content in ANCOVAs

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Table 2. Regression analysis between mean heterotrophy, α, of brown trout (*Salmo trutta*) (calculated from eq. 1) as independent variable and mean log concentration of the selected seven PCBs from the seven investigated rivers as dependent variables.

Dependent variable	df	Regression coefficient	r^2	p
PCB52 (dw)	6	1.00	0.46	0.095
PCB66/95 (dw)	6	1.00	0.40	0.130
PCB90/101 (dw)	6	0.99	0.44	0.110
PCB132/153/105 (dw)	6	0.85	0.56	0.052
PCB160/138/158 (dw)	6	0.96	0.55	0.057
PCB180 (dw)	6	0.91	0.59	0.044
PCB194 (dw)	6	1.00	0.56	0.052
PCB52 (lw)	6	1.25	0.63	0.033
PCB66/95 (lw)	6	1.25	0.58	0.048
PCB90/101 (lw)	6	1.24	0.63	0.033
PCB132/153/105 (lw)	6	1.10	0.77	0.010
PCB160/138/158 (lw)	6	1.21	0.73	0.014
PCB180 (lw)	6	1.16	0.76	0.010
PCB194 (lw)	6	1.25	0.68	0.022

Note: Three rivers were omitted, as α were >>1. df, regression coefficients, r^2 values, and p values are presented for each of the regression analyses with log concentrations of PCBs expressed on a dry weight (dw) basis and lipid weight (lw) basis.

were not significant, $p > 0.05$) (Fig. 2). Lipid content differed between the three trophic levels: trout ($n = 28$) had the highest, 11.0 ± 3.1%, followed by invertebrates ($n = 10$), 6.1 ± 2.9, and detritus ($n = 10$) and periphyton ($n = 10$) the lowest, 2.6 ± 1.2% and 0.9 ± 0.3%, respectively (ANOVA, df = 3,54, $p < 0.01$; Tukey–Kramer post hoc tests, $p < 0.05$) (Table 4). Trout length differed between the river sites (ANOVA, df = 9,18, $p < 0.01$). PCB concentrations were not related to individual trout length either on a dry weight or on a lipid weight basis (simple regressions, df = 27, $p > 0.05$).

On a dry weight basis, PCB concentrations generally increased with increasing trophic level, but when concentrations were expressed on a lipid weight basis, no increases were found (Fig. 3). The geometrical means of biomagnification factors for trout/invertebrates ($BMF_{trout/inv}$) for the different PCBs ranged from 0.6 to 3.7 on a dry weight basis and from 0.3 to 2.0 on a lipid weight basis. log $BMF_{trout/inv}$ showed a bell-shaped relationship with the log K_{OW} of the PCBs (Figs. 4*a* and 4*b*). On a dry weight basis, log $BMF_{trout/inv}$ for higher K_{OW} PCBs were >0 (Fig. 4*a*), while on a lipid weight basis, only one PCB was >0 and the two lower K_{OW} PCBs were <0 (Fig. 4*b*). The geometrical means of biomagnification factors for invertebrates/detritus ($BMF_{inv/detr}$) and invertebrates/periphyton ($BMF_{inv/per}$) for the different PCBs ranged from 2.7 to 5.9 for $BMF_{inv/detr}$ on a dry weight basis, from 1.1 to 2.3 for $BMF_{inv/detr}$ on a lipid weight basis, from 2.0 to 7.7 for $BMF_{inv/per}$ on a dry weight basis, and from 0.3 to 1.2 on a lipid weight basis. We found no relationships between either log $BMF_{inv/detr}$ or log $BMF_{inv/per}$ and log K_{OW} of the PCBs (Figs. 4*c* and 4*d*). On a dry weight basis, all log $BMF_{inv/detr}$ and log $BMF_{inv/per}$ were >0 except for log $BMF_{inv/per}$ for the two highest K_{OW} PCBs (Fig. 4*c*). On a lipid weight basis, only log $BMF_{inv/per}$ for the two highest K_{OW} PCBs were different from 0 (Fig. 4*d*).

Fig. 1. Regression analysis on the relationship between degree of heterotrophy, α, in age-0 brown trout (*Salmo trutta*) and log periphyton density in the rivers ($y = 1.7x - 0.8$, $r^2 = 0.88$, df = 5, $p < 0.01$). Each point represents the mean α of two or three trout from one river. Three sites where α >> 1 and one site where periphyton density could not be measured was excluded from the analysis.

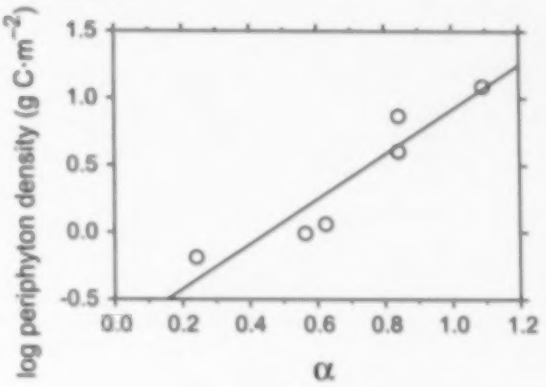
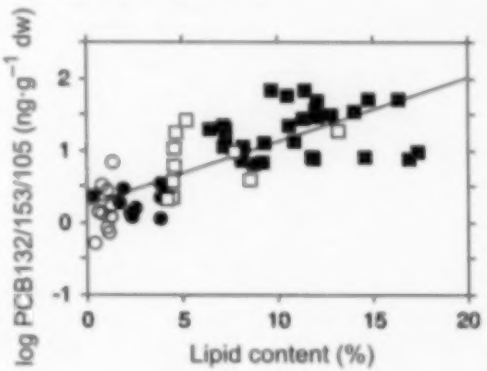


Fig. 2. Regression analysis between lipid content (% of dry weight) and log PCB132/153/105 concentrations in all the samples (solid circles, detritus of terrestrial origin; open circles, periphyton; open squares, invertebrates; solid squares, age-0 brown trout (*Salmo trutta*)) from the investigated rivers ($y = 8.9x + 0.24$, $r^2 = 0.59$, df = 57, $p < 0.01$).



The $\delta^{15}N$ ratios in the different compartments of the food webs varied between sites. At the base of the food webs, the $\delta^{15}N$ ratios in detritus ranged from 0.3‰ to 11‰, and periphyton $\delta^{15}N$ from 6.4‰ to 16‰. Also, trout $\delta^{15}N$ varied between sites, ranging from 7.5‰ to 19‰, although the within-site range was never more than 1.5‰ (Table 4). The within-site difference in $\delta^{15}N$ between trout and invertebrates was 2.6‰, which was significantly different from 0‰ but not from 3.4‰ (paired t tests, df = 9, with hypothesized differences = 0 and 3.4, respectively, and $p < 0.01$ and $p > 0.1$, respectively). Estimation of trophic position (eq. 2) gave a mean trophic position of 2.3 ± 0.8 for invertebrates and 3.1 ± 0.9 for trout. On a dry weight basis, PCB concentrations were positively related to the trophic position of all samples from all compartments (simple regressions, $r^2 = 0.11–0.48$, df = 57, $p < 0.05$) with regression coefficients

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Table 3. Differences in PCB concentrations expressed on a dry weight, lipid weight, and organic carbon weight basis between terrestrial primary producers (TPP), detritus of terrestrial origin (D), and periphyton (P).

	Dry weight			Lipid weight			Organic carbon weight		
PCB No.	TPP	D	P	TPP	D	P	TPP	D	P
52	—	—	—	<P	—	>TPP	<P	<P	>D >TPP
66/95	—	—	—	<P	—	>TPP	<P	<P	>D >TPP
90/101	—	—	—	<P	—	>TPP	<P	<P	>D >TPP
132/153/105	—	—	—	<P	—	>TPP	<P	<P	>D >TPP
160/138/158	—	—	—	<P	—	>TPP	<P	<P	>D >TPP
180	—	—	—	<P	<P	>D >TPP	<P	<P	>D >TPP
194	<P	<P	>D >TPP	<P	<P	>D >TPP	<P	<P	>D >TPP

Note: ">" or "<" denotes a significant difference between the different carbon sources and "—" denotes no significant difference (ANOVA, $df = 2,24$, Tukey–Kramer post hoc tests, $p < 0.05$).

Table 4. Means ± 1 SD and maximum–minimum (in parentheses) from the 10 investigated rivers of lipid content (% of dry weight), organic carbon content (% of dry weight), $\delta^{15}N$ (‰), $\delta^{13}C$ (‰), degree of heterotrophy (α), trophic position, and PCB concentrations (ng·g dry weight^{-1}) in the four investigated compartments: detritus of terrestrial origin ($n = 10$), periphyton ($n = 10$), invertebrates ($n = 10$), and age-0 brown trout (*Salmo trutta*) ($n = 28$).

	Detritus	Periphyton	Invertebrates	Trout
Lipids	2.6±1.2 (0.3–4.0)	0.9±0.3 (0.4–1.3)	6.1±2.9 (4.1–13.2)	11.0±3.1 (6.4–17.3)
Organic carbon	35±6.0 (28–45)	13±6.0 (6–22)	—	—
$\delta^{15}N$	6.3±3.5 (0.3–11.1)	10.2±2.9 (6.4–15.9)	10.8±3.0 (4.5–14.9)	13.2±2.6 (7.5–19.0)
$\delta^{13}C$	−28.7±1.0 (−30.0 to −27.0)	−27.3±5.5 (−33.9 to −17.2)	−28.5±1.3 (−31.5 to −27.0)	−28.1±1.9 (−32.3 to −25.7)
α	1	0	0.82±0.19 (0.63–1.03)	0.67±0.28 (0.23–1.11)
Trophic position	1	1	2.3±0.8 (1.3–3.6)	3.1±0.9 (2.0–5.0)
PCB52	0.9 (0.6–1.6)	0.7 (0.2–1.9)	4.7 (2.4–8.7)	2.9 (1.0–24.3)
PCB66/95	1.3 (0.6–2.6)	0.9 (0.2–3.8)	4.7 (1.3–20.5)	5.2 (1.3–45.2)
PCB90/101	1.3 (0.6–2.8)	0.9 (0.3–3.5)	5.3 (1.4–23.4)	7.6 (2.3–55.4)
PCB132/153/105	2.0 (1.2–3.5)	1.6 (0.5–7.1)	7.4 (2.1–26.3)	19.7 (6.6–70.6)
PCB160/138/158	2.4 (1.4–4.1)	1.8 (0.6–8.4)	8.1 (2.4–33.4)	20.0 (5.8–87.4)
PCB180	0.9 (0.6–1.6)	1.2 (0.3–6.3)	2.5 (0.7–12.0)	9.4 (2.8–34.1)
PCB194	0.1 (0.04–0.2)	0.2 (0.07–1.3)	0.5 (0.2–1.7)	1.3 (0.2–4.4)

and degree of explanation showing a bell-shaped relationship with the log K_{OW} of the compound. On a lipid weight basis, no such relationships were found. When invertebrates and trout were analyzed separately, no relationship was found between trophic position and PCB concentrations on either a dry weight basis or a lipid weight basis (simple regressions, $df = 9$ and $df = 27$, respectively, $p > 0.05$).

Discussion

Carbon source and PCB concentrations

We hypothesized that differences in carbon source between the systems would influence POP concentrations. The differences in PCB concentrations between terrestrial and aquatic primary producers in our study were low. In general, dry weight concentrations were similar; concentrations in periphyton were higher than in terrestrial primary producers on an organic carbon and lipid weight basis and also higher than in detritus of terrestrial origin on a lipid weight basis. However, the differences were small; ratios of terrestrial primary producers/periphyton concentrations were between 0.1 and 0.4. Therefore, any influence from the differences in carbon source on the PCB concentrations in the food chains is probably not from initial differences in concentrations between the terrestrial and aquatic primary producers.

A shift in carbon source or shift from heterotrophy to autotrophy is expected with an increase in nutrients and (or) an increase of stream order or watershed area or change in predation regime (Peterson et al. 1985; Finlay 2001; Nyström et al. 2003). With increased stream order or nutrient concentrations, autotrophy increases and α decreases. In the rivers of this study, the proportion of carbon ultimately derived from terrestrial sources, α, varied between 63% to 103% for invertebrates and between 23% to 111% for trout, indicating a higher utilization of autotrophic carbon sources by trout than by the invertebrates in our samples. The relative importance of terrestrial (allochthonous) versus aquatic (autochthonous) carbon sources to river food webs is highly variable between systems. The River Continuum Concept (Vannote et al. 1980) states that there is a longitudinal change from heterotrophic headwater streams to autotrophic midorder streams/rivers to heterotrophic large rivers, but the primary energy source supporting overall metazoan production in mid- to higher trophic levels of most rivers (fourth order and greater) may be autochthonous primary production entering food webs via algal–grazer pathways (Thorp and Delong 2002). In the rivers of this study, which fall into the category of headwater streams and midorder streams/rivers, the autochthonous production did not seem to be the primary energy source, as our mixed model (eq. 2) gave that carbon

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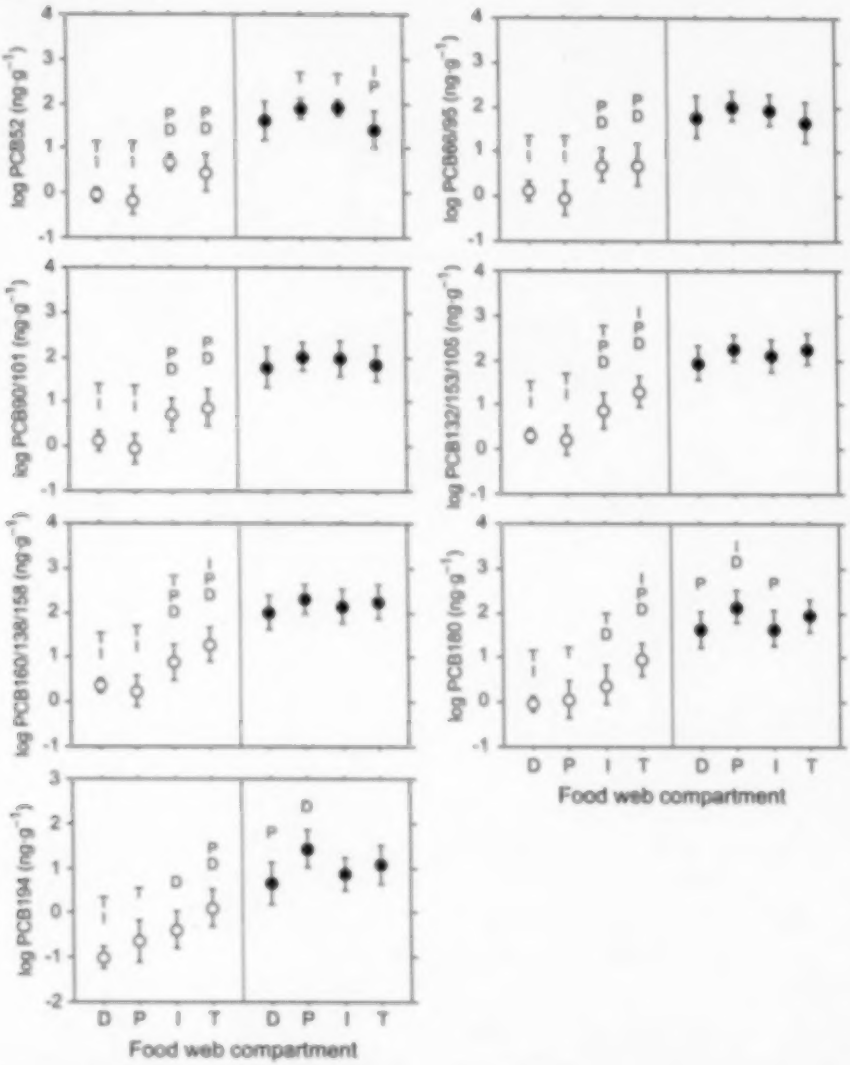
Fig. 3. Plots showing log concentrations of the seven PCBs in the four investigated compartments: detritus of terrestrial origin (D) (n = 10), periphyton (P) (n = 10), invertebrates (I) (n = 10), and age-0 brown trout (*Salmo trutta*) (T) (n = 28). Concentrations are expressed on a dry weight basis (open circles) and on a lipid weight basis (solid circles). Each point represents the mean and bars show ±1 SD. The letters above the boxes, D, P, I, and T, denote compartments with significantly different concentrations (ANOVAs, Tukey–Kramer post hoc tests, $p < 0.05$).



of terrestrial origin made up on average 82% of the invertebrate carbon and 67% of the trout carbon. A reliance of terrestrial carbon in the invertebrate communities of this study is expected, as they were largely composed of the shredder *G. pulex*, a species that feeds mainly on leaf litter (Sutcliffe et al. 1981). However, in the largest river in the study, Höje å, the trout carbon was primarily derived from autochthonous sources, and terrestrial carbon contributed with as little as 23% (but with 77% in the invertebrates). Thus, the relative influence of terrestrial and algal carbon sources in the streams and rivers in this study seems to follow the River Continuum Concept. The estimations of α resulted in unrealistic values (α >> 1) for invertebrate samples from five of the rivers and for trout from three of the rivers. These unrealistic values were produced when $\delta^{13}C$ values of the detritus of terrestrial origin and periphyton were either lower (in all cases but two) or higher than of the consumers. The variability of $\delta^{13}C$ in periphyton is known to be very high, and in many cases, it may be impossible for stable isotope analysis to discriminate between allochthonous and autochthonous carbon dependency in stream ecosystems (Nyström et al. 2003).

Contrary to our hypothesis, PCB concentrations in trout were positively related to α or increasing degree of heterotrophy. This may be due to an increased retention of PCB in the rivers caused by a higher density of periphyton (Fig. 5). With increasing autotrophy or reliance of periphyton as carbon source of the food webs, the density of periphyton de-

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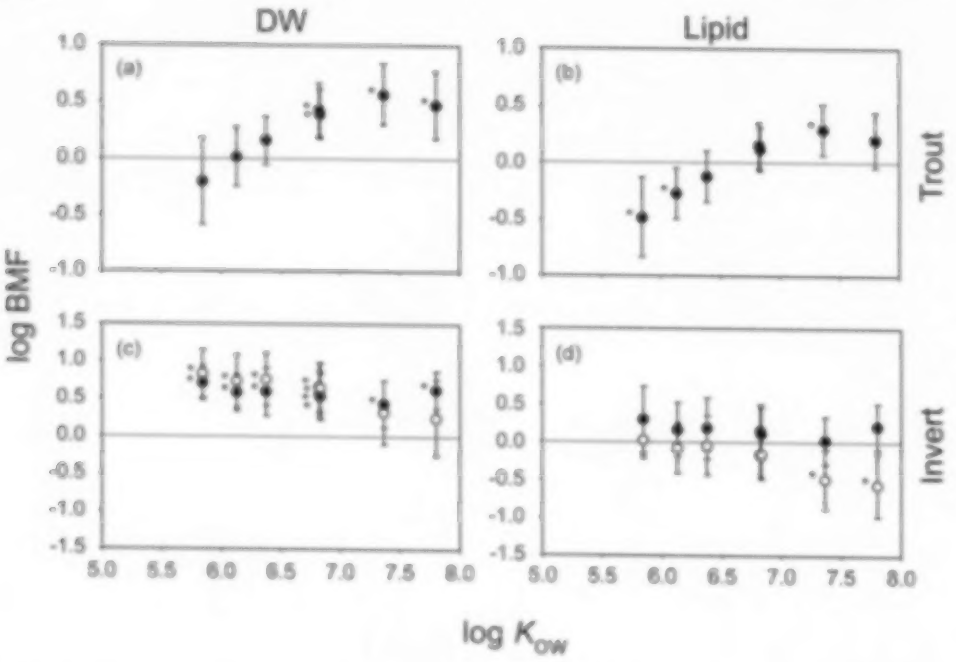
Fig. 4. (*a* and *b*) Relationships between the log octanol–water partitioning coefficients (K_{OW}) of the seven PCBs and log biomagnification factors (BMFs) between age-0 brown trout (*Salmo trutta*) and invertebrates expressed on a dry weight basis (Fig. 4*a*) and lipid weight basis (Fig. 4*b*). (*c* and *d*) Relationships between the K_{OW} of the seven PCBs and log BMFs between invertebrates and periphyton (open circles) and between invertebrates and detritus (solid circles) expressed on a dry weight basis (Fig. 4*c*) and on a lipid weight basis (Fig. 4*d*). Each point in all figures represents the mean BMF of the 10 rivers investigated ($n = 10$ for $BMF_{trout/inv}$, $BMF_{inv/per}$, and $BMF_{inv/det}$). Bars show ±1 SD. *Significant difference of log BMFs from 0 (one-sample *t* test with hypothesized difference = 0, $\alpha = 0.05$).



creases owing to increased grazing (Nyström and McIntosh 2003). Periphyton density in rivers is influenced by nutrients, light, stream velocity, and grazing (Rosemond et al. 2000). The relative importance of these factors may vary, but nutrient limitation is of less importance in rivers of this nutrient concentration range (Chételat et al. 1999). In the rivers in this study, we found no influence of nutrients, light, or stream velocity on periphyton biomass (Berglund 2003); however, we could not directly estimate the influence of grazing pressure in this study, as our sampling of invertebrates was not quantitative. POP concentrations in river biota have been shown to increase with increasing periphyton density (Berglund 2003). An increased periphyton density in the river will increase retention of POPs and increase the probability of uptake in biota, as predicted by the spiraling theory (Newbold et al. 1983; Stewart et al. 1993). The spiraling of compounds in rivers with unidirectional flow of water combines cycling with retention and accumulation in the ecosystem (Essington and Carpenter 2000). The retention increases with increasing periphyton density, which constitutes a large immobile surface area with which the POPs can associate, decreasing their downward transport and increasing the probability of uptake in biota. In lakes, an increase in primary producer biomass has been suggested to lower POP concentrations by biomass dilution (Taylor et al. 1991), but no such effect was found in these rivers (Berglund 2003). When the effect of periphyton density was removed, the correlation between α and PCB in this study disappeared. Thus, the carbon source or degree of heterotrophy/autotrophy may influence PCB concentrations in river food webs, but contrary to our hypothesis, the concentrations did not increase with increasing autotrophy owing to higher concentrations in aquatic primary producers than in terrestrial primary producers. Instead, concentrations decreased with increasing autotrophy, possibly as an indirect effect of increased grazing of periphyton that reduces periphyton biomass and thus reduces retention and uptake of PCBs in the river food web (Fig. 5).

Trophic position and stable isotopes

The between-site variation of $\delta^{15}N$ and $\delta^{13}C$ was high in all compartments including the bases, detritus and periphyton, of the food webs, an observation made in several stream studies (France 1995). The range in $\delta^{15}N$ was around 10‰ in each compartment, which would represent more than two trophic levels assuming an enrichment of 3‰–4‰ between trophic levels. The within-site variation in age-0 trout was low, however; the mean range was 0.7‰ and never exceeded 1.7‰. The compartment signatures of $\delta^{13}C$ also varied between sites. The variation was greatest within the aquatic primary producer compartment of the food chain as observed in several other studies (e.g., France 1995; Finlay 2001). Periphyton $\delta^{13}C$ ranged from –34‰ to –17‰, but, interestingly, that of terrestrial primary producers also varied

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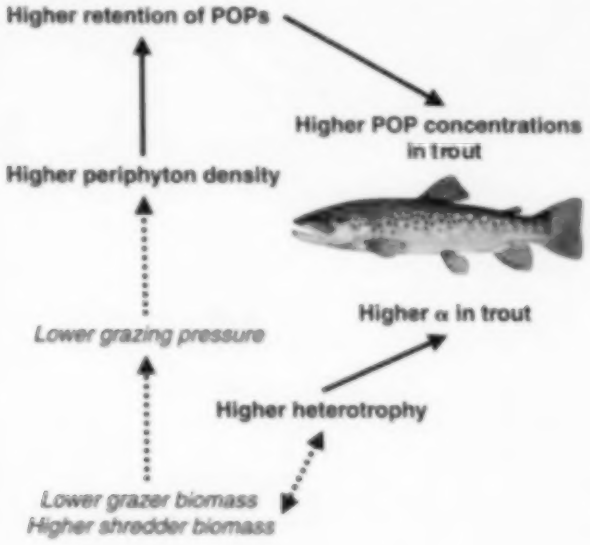
Fig. 5. Conceptual figure of the relationships between the benthic functional groups, degree of heterotrophy, α, and POP concentrations in brown trout (*Salmo trutta*). Broken arrows indicate relationships not explicitly tested in this study. The higher POP concentrations in trout with higher α may be indirectly caused by an increase of the heterotrophy/autotrophy ratio in the system. A decrease of the grazer/shredder ratio and decreased grazing pressure may lead to higher periphyton density, higher retention of POPs, and higher POP concentrations in trout as demonstrated by Berglund (2003).



considerably, between –32‰ and –26‰ (results not shown). Apparently, spatial variation in isotopic signatures can be substantial even within a fairly restricted geographical area like in this study (5000 km^2). To apply a stable isotope technique to multiple-system food webs like the rivers in this study, one requires obtaining an isotopic baseline at the base of the food webs in each system. In this study, we used the equations developed by Post (2002) to calculate trophic positions in a two-source food web where we used periphyton and detritus of terrestrial origin as the bases of the two food webs. To integrate temporal variation in isotopic signatures, Post (2002) recommended using primary consumers, and preferably long-lived, to quantify isotopic baselines. We chose to use primary producers and detritus, as there were no two distinct groups of primary consumers representing the autotrophic and heterotrophic food webs at all sites and also our chosen food webs had a low number of theoretical trophic levels with age-0 trout as our top predator. However, as the differences in age were small between the organisms in our compartments, temporal variation should not differ considerably between compartments in our food webs.

The mean estimated trophic position of all sites was 2.3 for invertebrates and 3.1 for age-0 trout, which indicates that the isotopic baseline quantification from eq. 2 was valid. The discrete trophic-level classification would predict invertebrates as primary consumers (level 2) and age-0 trout as secondary consumers (level 3). It is also reasonable for invertebrates to have a trophic position slightly higher than 2 and age-0 trout higher than 3, as a few invertebrates (leaches) in our samples are considered predatory (i.e., secondary consumers). However, the between-site variation of the estimated trophic position was high. The trophic position of invertebrates ranged from 1.3 to 3.6 and of age-0 trout from 2.0 to 5.0. It seems unlikely that age-0 trout would occupy trophic level 5 of the food web. Hence, the model seemed to estimate mean trophic positions using all sites satisfactory, but the sample sizes of the base of the food chain were probably too small ($n = 1$) to give reliable absolute estimates at each individual site.

PCB concentrations and food chain bioaccumulation

The support for food chain bioaccumulation of PCBs in these benthic, low trophic levels in rivers was not conclusive. On a dry weight basis, the PCBs with log K_{OW} values between 6.5 and 7.5 increased in concentration with the trophic position of the organisms in our river food webs. Mean concentrations of the seven selected PCBs were similar in detritus and periphyton and increased in invertebrates and the concentrations of PCB132/153/105, PCB160/138/158, and PCB180 were highest in age-0 trout. The concentrations of the seven selected PCBs in the samples were also positively related to the trophic position of the samples estimated using the data from the stable isotope analyses. Here, also, the slope and strength of the relationship were highest for PCB132/153/105, PCB160/138/158, and PCB180. However, this pattern was only observed when PCB concentrations were expressed on a dry weight basis. When concentrations were expressed on a lipid weight basis, no increase of PCB concentrations with trophic position was found. Lipid content explained 26%–68% of the variation in PCB concentrations (nanograms per gram dry weight) in all samples. We did not find relationships between lipid content and PCB concentrations within each compartment of the food webs. However, several biotic and abiotic factors differing between the river sites may affect the PCB concentrations in both water and organisms and therefore confound the lipid–PCB relationships (Berglund 2003), even though the input of PCBs to the river watersheds should be similar, as atmospheric deposition is similar over the region (Backe et al. 2000). Lipid content is one of the most important factors in explaining uptake of lipophilic POPs like PCBs in aquatic organisms, and lipid weight based concentrations are used when comparing accumulation in different organisms or systems, as the relative difference among lipid weight based concentrations is a good approximation of the relative differences in fugacity (Kucklick and Baker 1998; Mackay and Fraser 2000). The lipid content increased in the river food chains, from primary producers to primary consumers, and the highest lipid content was found in the predators, which has been proposed to be one of the factors explaining increasing levels of POPs in aquatic food chains (LeBlanc 1995). Therefore, we chose to use lipid weight based PCB concentrations in our food chains to examine food chain bioaccumulation and GI magnification. Consequently, PCB concentrations expressed on a dry weight basis increased with trophic level both when expressed with discrete levels and when calculated on a continuous scale from $\delta^{15}N$ and $\delta^{13}C$, but when expressed as nanograms per gram lipid, no relationship was found between PCB concentration and trophic level. Thus,

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the increasing lipid content in the food chain could explain the apparent increase of concentration with increasing organism trophic position.

To investigate steady-state biomagnification caused by GI magnification, we calculated biomagnification factors for each trophic transfer by dividing the concentration in the consumer with the concentration in the putative food. In this study, biomagnification factors for the seven selected PCBs between invertebrates and detritus ($BMF_{inv/det}$) or periphyton ($BMF_{inv/per}$) were between 0.3 and 2 on a lipid weight basis, and biomagnification factors in the second trophic transfer between trout and invertebrates ($BMF_{trout/inv}$) ranged from 0.3 to 2.0 on a lipid weight basis. Only PCB180 had biomagnification factors higher than 1, and PCB52 and PCB66/95 had biomagnification factors lower than 1. These biomagnification factors are low compared with other studies on piscivorous fish (3–5; Russell et al. 1995) or planktivorous fish (1–3; Hoekstra et al. 2003). However, the results are similar to those found in planktonic food chains in lakes (Berglund et al. 2000) where there was no biomagnification in the first trophic transfer between zooplankton and phytoplankton and only an increase (biomagnification factors = 1.5–3.0) in concentrations of the PCBs with log $K_{OW} > 6.5$ in the second trophic transfer between zooplankton and planktivorous young-of-the-year roach (*Rutilus rutilus*). Thus, PCBs were magnified on a dry weight basis but not on a lipid weight basis in the first trophic transfer between periphyton and detritus, and invertebrates and the biomagnification factors were independent of the K_{OW} values of the PCBs. Hence, the accumulation in invertebrates corresponds to an equilibrium partitioning of PCBs either with food or with water. The biomagnification factors in the second trophic transfer were related to the K_{OW} of the compounds. Biomagnification factors were lower than 1 for PCBs with log $K_{OW} < 6.5$ and higher than or not different from 1 for PCBs with log $K_{OW} > 6.5$. Therefore, equilibrium partitioning with food cannot explain the accumulation in age-0 trout. According to the GI magnification model (Gobas et al. 1999), chemicals with a log $K_{OW} < 5$ will not magnify from food to consumer owing to a high rate of chemical elimination, and Sijm et al. (1992) showed that the elimination rates decreased with increasing size or decreasing growth rate of the consumer. The biomagnification factors <1 indicate that age-0 trout are not in equilibrium or steady state with their environment, which may be caused by high growth dilution owing to high growth rates, and the biomagnification factors >1 also indicate nonequilibrium, which may be caused by GI magnification. A GI magnification at non-steady-state conditions may thus explain the shape of the $BMF_{trout/inv}$–K_{OW} relationship. The fact that lipid content did not explain PCB concentrations of organisms within each compartment of the food chain could suggest that equilibrium or steady state is not reached between the organisms and the environment or the food in the rivers of this study. It seems that to observe food chain accumulation as a result of GI magnification, organisms with lower growth rates (i.e., closer to steady state) should be studied.

We used PCB concentrations on a lipid weight basis when testing biomagnification and calculating biomagnification factors in all compartments of the food chain. Although it is often assumed that in plants and algae, POP accumulation is a passive process governed by the compounds' affinity for the lipids of plant and algae, controversy remains with regard to the importance of the lipid fraction as the accumulation site of POPs in the biota, especially for plants and algae (Skoglund and Swackhamer 1999). Rather than the lipid fraction, the organic carbon fraction is often used to normalize POPs in plants and algae (Skoglund and Swackhamer 1999). If other nonlipid organic materials play an important role in the accumulation of POPs in primary producers, normalization to lipids may lead to overestimations of the primary producer concentrations entered in the biomagnification factor calculations. In our study, the lipid content and organic carbon content in periphyton and detritus were related ($f_{oC} = 0.1 + 9f_{lip}$, $r^2 = 0.75$). Thus, the organic carbon fraction (f_{oC}) was one order of magnitude higher than the lipid fraction (f_{lip}) in detritus and periphyton. However, like lipids, the organic carbon fraction could not predict PCB concentrations within each of the two compartments in this study. Lipid content has been shown to accurately predict PCB accumulation in both fish and invertebrates (Kucklick and Baker 1998; Berglund et al. 2000). Therefore, we used dry matter and lipid rather than carbon-normalized PCB concentrations for all compartments when testing food chain bioaccumulation and biomagnification factors in our food chains.

In conclusion, several ecological and chemical factors influence the accumulation of POPs in aquatic organisms. The degree of heterotrophy/autotrophy in rivers may influence accumulation of POPs through indirect effects from the dominating functional groups of the benthic community. Contrary to what we predicted in this study, a high degree of autotrophy decreased PCB concentrations in age-0 trout. The high grazing pressure in the algae/grazer-dominated autotrophic systems may suppress periphyton densities, thereby reducing the retention and exposure of lipophilic contaminants to the river biota.

Concentrations of POPs in aquatic food webs, and in particular at the lower trophic levels with organisms of similar size and age, are not determined only by the trophic position of the organisms. In this study, lipid content was the best predictor of PCB concentrations in the food chain periphyton/detritus – invertebrates – age-0 trout, and lipid weight based concentrations did not increase with trophic position. However, lipid content could not predict PCB concentrations within each trophic level, and on a dry weight basis, PCB concentrations increased with trophic position. We did find indications of GI magnification of PCBs in age-0 trout but not in invertebrates. Possibly, the age-0 trout in this study were not in steady state with their environment owing to high growth rates, probably higher than for fish used in other GI magnification and food chain bioaccumulation studies.

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Periphyton response to long-term nutrient enrichment in a shaded headwater stream

Jennifer L. Greenwood and Amy D. Rosemond

Abstract: We maintained elevated but moderate concentrations of nitrogen and phosphorus continuously for 2 years in a heavily shaded headwater stream and compared effects on stream periphyton with a reference stream. Both streams were sampled for 1 year before treatment. Some measures of periphyton biomass (ash-free dry mass and chlorophyll *a*) responded positively to enrichment. Increased chlorophyll *a* was likely a result of higher chlorophyll per cell, as total algal biovolume did not change with enrichment. These differences were greatest during high-light months (November–May), when cellular growth rates (a proxy for production) were also highest with enrichment. Algal assemblages were dominated by diatoms and remained similar between the treatment and reference streams throughout the enrichment period. Although nutrients stimulated algal growth rates, the long-term effects of nutrient addition on periphyton biomass were small in magnitude compared with other published values and were potentially suppressed by light availability and invertebrate consumption. These and other factors may have also been important in limiting the algal species pool and thus a taxonomic response to enrichment. Our results indicate that in headwater streams with intact tree canopies, chronic nutrient enrichment at moderate concentrations may have little detectable effect on benthic algal composition or periphyton biomass. Although nutrients stimulated algal growth rates, the long-term effects of nutrient addition on periphyton biomass were small in magnitude compared with other published values and were potentially suppressed by light availability and invertebrate consumption. These and other factors may have also been important in limiting the algal species pool and thus a taxonomic response to enrichment. Our results indicate that in headwater streams with intact tree canopies, chronic nutrient enrichment at moderate concentrations may have little detectable effect on benthic algal composition or periphyton biomass.

Résumé : Pendant deux années complètes, nous avons maintenu modérément élevées les concentrations d'azote et de phosphore d'un ruisseau de tête de bassin fortement ombragé; nous avons comparé les effets sur le périphyton de ce ruisseau aux conditions observées dans un ruisseau témoin. Les deux ruisseaux ont aussi été échantillonnés pendant un an avant le traitement expérimental. Certaines valeurs de biomasse du périphyton (masse sèche sans les cendres et chlorophylle *a*) augmentent après l'enrichissement. L'augmentation de chlorophylle *a* est vraisemblablement due à un contenu plus élevé de chlorophylle par cellule, car le biovolume total des algues ne change pas avec l'enrichissement. Ces différences sont plus marquées durant les mois de forte lumière (novembre à mai) pendant lesquels les taux de croissance cellulaire (une valeur de remplacement de la productivité) sont aussi maximaux dans les conditions d'enrichissement. Les peuplements d'algues sont dominés par les diatomées et sont restés semblables dans le ruisseau expérimental et le ruisseau témoin durant la durée de l'enrichissement. Bien que les nutriments stimulent les taux de croissance des algues, les effets à long terme de l'addition de nutriments sur le périphyton restent de faible amplitude par comparaison à d'autres valeurs trouvées dans la littérature; il y a donc potentiellement une suppression des effets par la disponibilité de la lumière et la consommation des invertébrés. Ces facteurs et d'autres peuvent aussi être importants pour limiter le pool d'espèces d'algues et ainsi réduire la réponse taxonomique à l'enrichissement. Nos résultats indiquent que, dans les ruisseaux en tête de bassin où la couverture des arbres est intacte, un enrichissement chronique mais modéré en nutriments peut avoir peu d'effets décelables sur la composition des algues benthiques ou sur la biomasse du périphyton.

[Traduit par la Rédaction]

Introduction

Rivers play critical roles on the landscape in providing essential services to humans. Intact headwater streams are crucial to the functioning of river systems (Meyer and Wallace 2001) and have been shown to be critical sites in river networks for processes such as nutrient uptake and retention (Peterson et al. 2001). However, headwater streams are also particularly vulnerable to changing land use and non-point-source pollutants. In many cases, these streams are not pro-

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tected simply because they do not appear on maps or are not adequately protected by law (Meyer and Wallace 2001). Small-order streams also have a greater contribution of watershed area to stream area compared with larger streams (Selby 1985). Thus, first-order streams may experience greater nutrient inputs than larger streams owing to atmospheric deposition, saturation of terrestrial ecosystems, or mobilization from soils from the surrounding catchment. However, the effects of long-term nutrient input to forested headwater streams are essentially unknown.

Most experimental work examining effects of enrichment on periphyton has been short term in nature (less than ~8 weeks; Francoeur 2001) and thus demonstrates potential or transient responses rather than ultimate effects. The importance of long-term studies is evident from a 16-year study in the Kuparak River (Alaska, USA), where nutrient addition during the growing season each year resulted in consistently higher algal biomass in a fertilized versus reference reach of stream (Slavik et al. 2004). The effects of enrichment were greatest during the first 2 years of enrichment but were suppressed by grazers in subsequent years (Miller et al. 1992; Peterson et al. 1993). Further, a dramatic increase in bryophyte cover after 8 years of enrichment reduced the magnitude of enrichment effects on epilithic algal biomass, but epiphytic algae associated with bryophytes exhibited a strong positive response to enrichment (Slavik et al. 2004). The high-light environment of a tundra stream like the Kuparak contrasts with many headwater streams that are heavily shaded. Short-term experimental work in forested headwater streams indicates that the response of algal biomass to nutrient enrichment can be limited by light availability and invertebrate consumption (e.g., Lowe et al. 1986; Rosemond 1993; Bernhardt and Likens 2004). In environments where response to enrichment is predicted to be subtle or suppressed by other factors (systems with heavy shade or where algal biomass is controlled by grazers), longer-term studies are critical for predicting the potential ultimate effects of nutrients on periphyton structure and function.

Fewer data exist to make clear predictions regarding the expected response of algal assemblages to nutrient enrichment. Algal assemblages are often sensitive to changes in water chemistry because of different tolerance optima among individual populations (Lowe 1974) or differences in competitive ability among species (Tilman 1977), and the great majority of studies that have examined species assemblage response to elevated nutrient concentrations have found significant shifts in taxonomic composition (e.g., Fairchild et al. 1985; Marks and Lowe 1989; Mulholland and Rosemond 1992). However, community shifts are difficult to predict, since they do not always change with nutrient enrichment, regardless of the biomass response (Shortreed et al. 1984; Lohman et al. 1991; Peterson et al. 1993). Specifically, in some situations, other factors (i.e., stressful or poor growing conditions, intense grazing) can be more important than nutrients in driving variation in assemblage composition and can minimize the capacity of species assemblages to respond to nutrient enrichment (Hill et al. 1992; Rosemond et al. 2000).

Our study examined periphyton response to long-term nutrient addition in a shaded headwater stream. We determined the effects of a 2-year continuous enrichment of nitrogen and phosphorus on several characteristics of epilithic periphyton assemblages. We measured algal biomass as ash-free dry mass (AFDM) and chlorophyll *a*, determined algal species composition, and assessed algal growth rates as a proxy measure of productivity. We hypothesized that because of light limitation, nutrient enrichment would have little overall effect on algal biomass and cellular growth rates of benthic algae would be constrained. However, because of variation in nutrient optima among algal taxa, we predicted that algal assemblage structure would be altered with changes in the relative abundance of common species.

Materials and methods

Study site

Two headwater streams, one enriched and one serving as a reference, were examined for 3 years (July 1999 – July 2002) at the Coweeta Hydrologic Laboratory, a USDA Forest Service research facility located in the Blue Ridge Mountain physiographic province in the southern Appalachian Mountains (North Carolina, USA). The reference stream (Catchment 53) and the treatment stream (Catchment 54) have similar physical and chemical characteristics (Table 1). Dominant vegetation includes tulip poplar (*Liriodendron tulipifera*), white oak (*Quercus alba*), red oak (*Quercus rubra*), red maple (*Acer rubrum*), and dogwood (*Cornus florida*) (Swank and Crossley 1988). Rhododendron (*Rhododendron maximum*), an evergreen, grows as a dense understory in the riparian zone. Thus, a double canopy of deciduous trees and rhododendron shades the study streams to some degree during all seasons.

Nutrient enrichment

Pretreatment data were collected from both streams from July 1999 to July 2000. Beginning 11 July 2000, the treatment stream was continuously enriched with NH_4NO_3, KH_2PO_4, and K_2HPO_4 along the entire 150-m length of the study reach for 2 years. A dissolved nutrient salt solution was pumped into an irrigation line (~2 cm in diameter) that was fed with stream water from an upstream head tank. The line ran the entire length of study reach adjacent to the streambed with nutrient solution delivered from multiple spigots. A metering pump (Liquid Metronics, Inc., Acton, Mass.) was electronically linked with a Campbell data logger to an Isco (Teledyne Isco, Los Angeles, Calif.) flow measurement device at the downstream end of the stream reach to deliver nutrients in a discharge-dependent manner. Stream water nutrient concentrations were measured during the pretreatment and experimental periods. During the pretreatment period, one to four samples were taken from the reference and treatment streams on five sampling dates in the reference stream and 12 sampling dates in the treatment stream. During the enrichment period, one sample was taken from the reference stream and five samples were taken along the length of the treatment stream approximately every 2 weeks to confirm that nutrients were elevated in an even distribution within the study reach. Stream water samples were filtered with Millipore HA filters (Millipore Corp., Billerica, Mass.) into acid-washed bottles and frozen until analysis. Concentrations of NO_2^--N, NO_3^--N, NH_4^+-N, and soluble reactive phosphorus (SRP) were determined with an

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Table 1. Physical and chemical characteristics of the reference stream and enriched stream from the Coweeta Hydrologic Laboratory.

		Reference	Treatment
Catchment	Area (ha)	5.2	5.5
	Elevation (m above sea level)	820	841
Channel	Gradient ($cm \cdot m^{-1}$)	27	33
	Length (m)	145	282
	Bankfull area (m^2)	327	443
Temperature (°C)	Daily mean (n)	12.0 (336)	12.0 (336)
	Range	2.6–18.6	4.8–16.7
Discharge ($L \cdot s^{-1}$)	Daily mean (n)	0.32 (1114)	0.53 (1114)
	Range	0.006–3.8	0.06–4.8
pH	Mean (n)	6.59 (24)	6.87 (18)
	Range	6.2–7.0	6.6–7.9
NO_3-N ($\mu g \cdot L^{-1}$)	Mean (n)	15.4 (5)	18.8 (12)
	Range	9.4–25.8	4.0–39.5
NH_4-N ($\mu g \cdot L^{-1}$)	Mean (n)	9.4 (4)	9.9 (12)
	Range	0–30.4	0–24.9
SRP ($\mu g \cdot L^{-1}$)	Mean (n)	7.6 (5)	8.8 (12)
	Range	0–20.3	0–22.1

Note: Discharge and temperature are from July 1999 to July 2002 and nutrient and pH data are from the pretreatment period July 1999 – July 2000. SRP, soluble reactive phosphorus.

Alpkem Rapid Flow Analyzer 300 (Alpkem, College Station, Texas) at the University of Georgia Chemical Analysis Laboratory (Athens, Georgia).

Biomass

Epilithic algae were sampled from unglazed ceramic tiles (5.3 cm^2) every 2 months on approximately the 15th of the month from July 1999 through July 2002. Tiles were colonized for 2 months prior to collection (i.e., when tiles were collected, they were replaced with uncolonized tiles that were collected 2 months later). Five sets of two tiles were secured to the streambed along the length of each stream. Accumulated debris (particularly allochthonous leaf material) was cleared from tiles at least once per week. One tile was used for determination of AFDM and the other for chlorophyll *a*. Periphyton used for AFDM samples was brushed from the tile with a toothbrush, rinsed, and the resulting slurry filtered onto a preashed Gelman A/E glass fiber filter (Pall Life Sciences, Ann Arbor, Mich.). The filters were weighed before and after combusting at 500 °C to determine AFDM. Chlorophyll *a* was measured by extracting colonized tiles directly in 20 mL of 90% alkaline (with NH_4OH) acetone solution in a freezer for 24 h. Chlorophyll *a* content was measured with a Turner model 112 fluorometer (July 1999 – May 2001) and a Turner TD-700 fluorometer (July 2001 – July 2002) (Turner Biosystems Inc., Sunnyvale, California). Photosynthetically active radiation (PAR) was measured starting July 1999 at the reference stream and November 1999 at the treatment stream at each sampling site during the study period with a LI-COR LI-250 model hand-held light meter (LI-COR, Linclon, Nebraska).

Growth rates

We sought to determine a measure of algal productive capacity between the two streams that was not influenced by potential losses owing to grazers and that also examined potential effects of irradiance on nutrient response. Algal productivity in these streams was too low to measure with an oxygen change method and measuring ^{14}C uptake was logistically problematic. Thus, we used algal cellular growth rates as a proxy for productive capacity by assessing accrual rates of algal cells on glass slides before and after canopy closure. This method also allowed us to measure algal accrual in the absence of grazers, which were excluded from experimental chambers. In-stream channels were deployed for 4 weeks during four separate trials in "March" (21 March – 4 April), "April" (18 April – 1 May), "June" (21 May – 5 June), and "July" (26 June – 10 July) 2002. In-stream channels were constructed using 30-cm lengths of vinyl rain gutter. To exclude macrograzers, we glued two layers of 200-µm Nitex mesh with silicon sealant to both ends of the gutter. No grazers were detected in the gutters during the experiment. Five gutters each were placed in the treatment and reference streams. Microscope slides were used as the colonization substratum. Three slides were glued horizontally into each channel with silicon sealant. One slide was collected after 2 weeks (2 weeks of accrual time) and another slide at 4 weeks (total of 4 weeks of accrual time) from each of the five gutters in each stream. Slides were scraped with a razorblade and fixed in 1 mL of 2.5% formalin. Cell densities were enumerated in a Palmer–Maloney cell as for species composition, below. New cell accrual between weeks 2 and 4 was calculated as the increase in number of cells per day, which was compared between each stream for each date. This time frame was adequate for distinguishing growth period (2- to 4-week accrual) from confounding factors of the colonization period (0- to 2-week accrual) and avoided the potential for longer-term biomass accrual obscuring differences in growth rates.

Species composition and biovolume

Beginning in September 1999, one additional tile was collected with each set of biomass tiles for algal taxonomic analysis. Tiles were brushed with a toothbrush, rinsed, and

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Table 2. Average pretreatment (July 1999 – July 2000) and treatment (July 2000 – July 2002) nutrient levels ($\mu g \cdot L^{-1}$) for the reference and treatment streams.

Site	$(NO_2^- + NO_3^-)$-N	NH_4^+-N	SRP
Pre-enrichment			
Reference			
Mean (1 SD)	15.4 (6.6)	9.4 (14.1)	7.6 (8.0)
Range (*n*)	9–26 (5)	BD–30 (5)	BD–20 (5)
Treatment			
Mean (1 SD)	18.8 (11.5)	9.9 (8.6)	8.8 (8.1)
Range (*n*)	4–40 (12)	BD–25 (12)	BD–22 (12)
Post-enrichment			
Reference			
Mean (1 SD)	16.9 (29.8)	10.4 (16.9)	3.7 (4.7)
Range (*n*)	BD–151 (33)	BD–76 (33)	BD–17 (33)
Treatment			
Mean (1 SD)	308.9 (377.8)	105.5 (119.7)	51.2 (55.6)
Range (*n*)	11–1711 (44)	6–566 (44)	BD–268 (44)

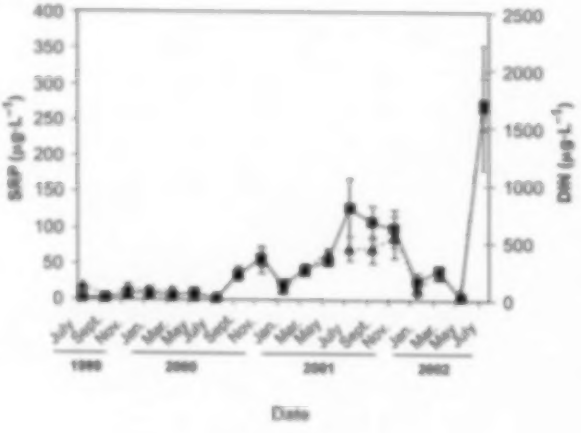
Note: SRP, soluble reactive phosphorus; BD, below detection.

the entire slurry fixed in a total of 20 mL of 2.5% formalin solution. Community composition and cell density were determined by counting at least 500 cells in a Palmer–Maloney nanoplankton counting chamber at 400× magnification. In cases where cell densities were too low to find 500 cells, 10 transects of the Palmer cell were examined. Diatom species were identified after cleaning with 30% H_2O_2 and $K_2Cr_2O_7$ to clear cell contents and permanently mounting in Naphrax high-resolution mounting medium. Species composition was assessed by identifying 300 complete frustules at 1000× magnification. In cases where cell densities were too low to find 300 frustules, 10 transects of the diatom slide were examined and all complete frustules found were identified. To calculate algal cell biovolume, we applied average dimensions of up to 10 cells in each taxon to standard geometric shapes that best represented the shape of each taxon (Hillebrand et al. 1999). Biovolumes from the Academy of Natural Sciences database from the Phycology Section of the Patrick Center for Environmental Research biovolume database (http://diatom.acnatsci.org/nawqa/2001biovol.asp) were used to estimate biovolumes for rarely encountered taxa.

Data analysis

Randomized intervention analysis (RIA) (Carpenter et al. 1989) was used to assess differences between the reference and treatments streams in AFDM, chlorophyll *a*, total biovolume, and relative biovolume of algal species with >5% average biovolume from tiles. RIA compares differences between unreplicated reference and treatment systems before and after an intervention (in this case, nutrient addition). A significant difference with RIA would result from an increase or decrease in the difference between reference and treatment streams. Appropriateness of parametric versus nonparametric tests for comparisons of seasonal levels of PAR and algal biomass and also growth rate data was determined by testing the data for normality by fitting to a normal distribution model. Owing to lack of fit with the normal distribution, Kruskall–Wallis with a Bonferroni correction for multiple tests (Ott and Longnecker 2001) and Tukey's multiple comparison tests were used to compare PAR and biomass data. Algal growth rates fit a normal distribution and differences between streams and individual trials were assessed with a two-way ANOVA and a Tukey test. Tests for normal distribution, Kruskall–Wallis, Tukey's, and the two-way ANOVA were performed with JMP (release 5.0.1a) (SAS Institute Inc., Cary, North Carolina).

Fig. 1. Stream water nutrient concentrations in the treatment stream ± 1 SE as soluble reactive phosphorus (SRP) (triangles) and dissolved inorganic nitrogen (DIN) (squares). Data points represent means from data collected during the previous 2 months.



Results

Nutrient enrichment

On average, the enrichment increased dissolved inorganic nitrogen approximately 13 times and SRP approximately five times to a long-term mean of ~400 µg NO_3^--N + NH_4^+-N·L^{-1} and 45 µg SRP·L^{-1} in the treatment stream (Fig. 1; Table 2). These nutrient concentrations are within the range of those found regionally in streams (Scott et al. 2003). Phosphorus and nitrogen varied during the enrichment period (Fig. 1) with decreased nutrient concentrations during early winter

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Fig. 2. Algal biomass ± 1 SE as (*a*) chlorophyll *a* and (*b*) ash-free dry mass (AFDM) from the reference stream (open circles and broken line) and treatment stream (solid circles and solid line) (randomized intervention analysis (RIA), $p < 0.05$ for both chlorophyll *a* and AFDM).

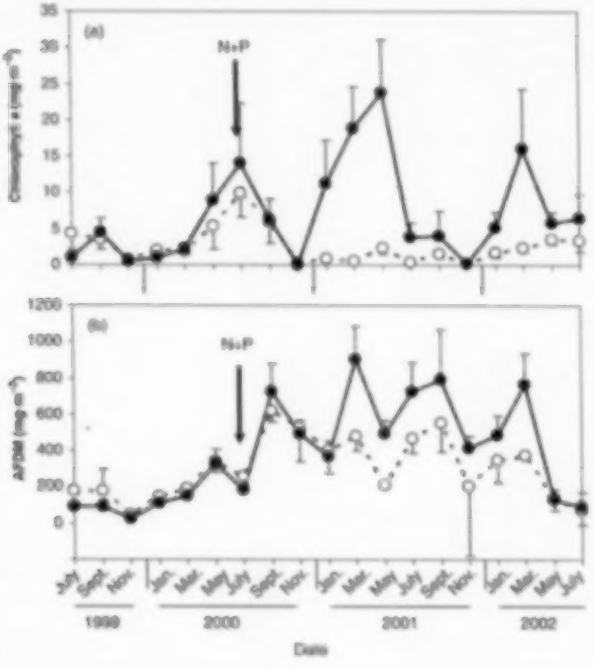
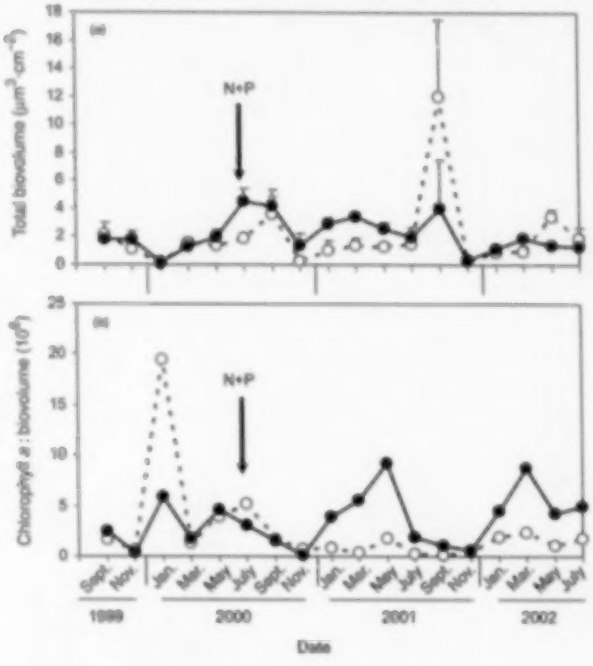


Fig. 3. Algal biomass ± 1 SE as (*a*) total biovolume and (*b*) chlorophyll *a* per biovolume from the reference stream (open circles and broken line) and treatment stream (solid circles and solid line). Randomized intervention analysis (RIA) showed that differences between streams before and after enrichment were not significantly different ($p > 0.05$).

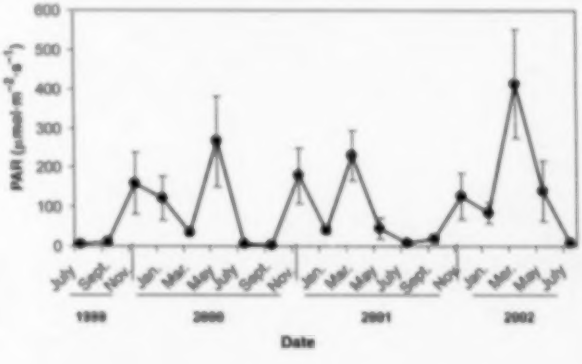


2001 and winter–spring 2002 that increased again in the summer and early fall of both years. Nutrient concentrations in the reference stream remained consistent across pretreatment and treatment periods with total inorganic nitrogen <30 $\mu g \cdot L^{-1}$ and SRP < 5 $\mu g \cdot L^{-1}$. The mean nitrogen to phosphorus ratio of stream water collected from the treatment stream over the enrichment period was 19.7. This ratio contrasts with the 11.1 ratio that was added in our stock solution, indicating greater in-stream uptake of phosphorus relative to nitrogen.

Biomass response

Periphyton biomass, as both AFDM (Fig. 2*b*) and chlorophyll *a* (Fig. 2*a*), was significantly greater in the treatment stream relative to the reference stream after nutrient enrichment (RIA, $p < 0.05$). Chlorophyll *a* was typically 2–9 $mg \cdot m^{-2}$ prior to enrichment and increased to 23 $mg \cdot m^{-2}$ in the treatment stream in year 1 (in May) of enrichment but only up to 17 $mg \cdot m^{-2}$ in year 2 (in March). Pretreatment values for ADFM were low and similar in the two streams (100–300 $mg \cdot m^{-2}$), and although AFDM in both streams was higher in the period after July 2000, AFDM was roughly 50% greater in the treatment versus reference stream beginning in March 2001 (up to 900 $mg \cdot m^{-2}$). Chlorophyll *a* showed a greater increase during the spring of each year compared with summer and fall. Increased AFDM in the treatment stream showed no such seasonal effect. There was very little difference in either AFDM or chlorophyll *a* between treatment and reference streams on our last two sampling dates in summer 2002.

Fig. 4. Photosynthetically active radiation (PAR) ± 1 SE averaged from the treatment stream ($n = 5$) and reference stream ($n = 5$).



Algal biomass as total biovolume (Fig. 3*a*) was not significantly different between streams after nutrient enrichment (RIA, $p > 0.05$). Biovolume exhibited a slight seasonal pattern where biovolume was lowest, $<2 \times 10^6$ $\mu m^3 \cdot cm^{-2}$, during November or January and reached a maximum of $\sim 4 \times 10^6$ $\mu m^3 \cdot cm^{-2}$ in mid- to late summer. An extremely high value for biovolume in the reference stream after enrichment

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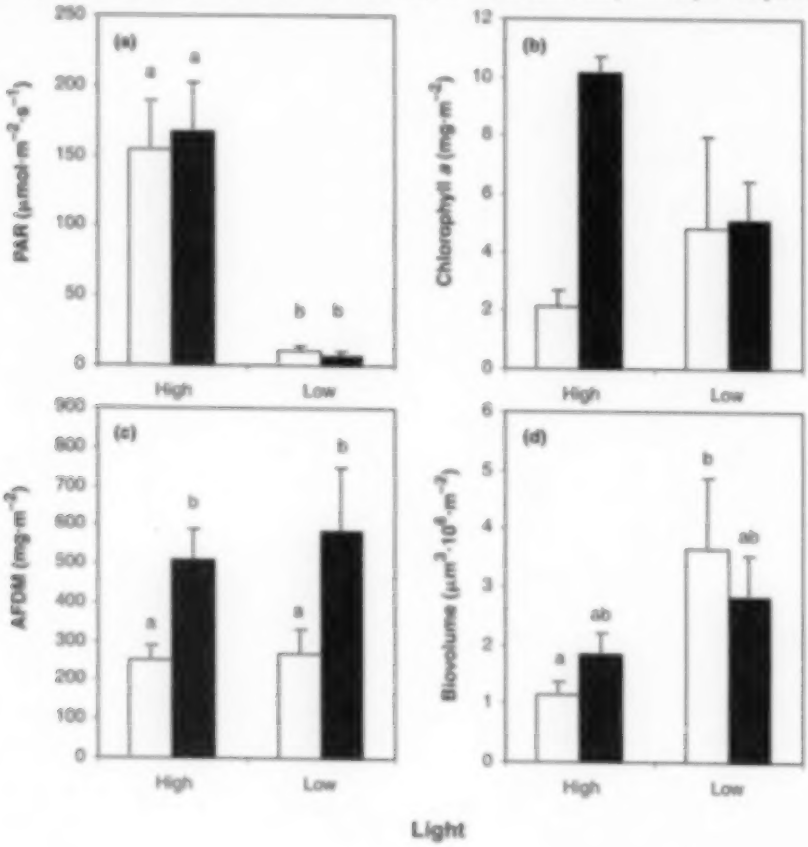
Fig. 5. Average (*a*) photosynthetically active radiation (PAR), (*b*) chlorophyll *a*, (*c*) ash-free dry mass (AFDM), and (*d*) total biovolume ± 1 SE in the reference stream (open bars) and treatment stream (solid bars) during seasonal periods of high and low light availability. Different letters above the bars indicate Kruskal–Wallis at $p < 0.0125$ and Tukey's multiple comparison at $p < 0.05$.



was associated with one replicate having unusually high algal accrual. The ratio of chlorophyll *a* to biovolume (Fig. 3*b*) contained a very high value in the reference stream prior to enrichment owing to a very low biovolume value (0.1×10^6). This data point was considered spurious and was removed before RIA. Thus, there was also no significant difference between streams in the ratio of chlorophyll *a* to biovolume (RIA, $p > 0.05$) (Fig. 3*b*). However, there was a trend of higher values during the spring months in the treatment stream, as was seen for chlorophyll *a*.

Relationships between periphyton biomass and irradiance

Instantaneous PAR levels showed seasonal trends, with lowest levels in July and September and seasonal peaks in November and in May the first year and in March the next 2 years (Fig. 4) (light data were lost for July 2000 and January 2001 in the treatment stream because of instrument malfunction). To determine whether variation in periphyton response to nutrients was due to season, we compared average light levels during periods of higher (January, March, May, and November) and lower (July and September) light and then compared algal biomass between treatment and reference streams during these time periods. Regressions run between biomass measurements and light levels with and without enrichment were nonsignificant ($p > 0.05$) with little variation explained by the model ($r^2 < 0.05$), most likely because of the variability inherent in instantaneous light readings. Thus, we decided to use longer-term averages to assess the relationship between seasonal light and chlorophyll *a* levels. Data from the treatment stream during pretreatment were included with values for the reference stream. Instantaneous PAR levels averaged ~150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during November–May and 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during July–September (Fig. 5*a*). Light levels were significantly (Bonferroni-corrected significance value = 0.0125) higher during November–May in both streams compared with July–September (Kruskal–Wallis, $\chi^2 = 19.16$, df = 3, $p < 0.0005$; Tukey's multiple comparison, $p < 0.05$). Chlorophyll *a* levels were not significantly different between streams but peaked overall in the treatment stream during November–May (Kruskal–Wallis, $\chi^2 = 7.85$, df = 3, $p = 0.05$) (Fig. 5*b*). AFDM levels were significantly higher in the treatment versus the reference stream during both periods (Kruskal–Wallis, $\chi^2 = 11.22$, df = 3, $p < 0.01$; Tukey's multiple comparison, $p < 0.05$) (Fig. 5*c*). Biovolume was significantly higher only in the reference

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Table 3. *F* values from two-way ANOVA of log algal cell accrual rate.

Treatment	df	Accrual rate
Nutrient	1	5.58* (enriched stream > reference)
Month	3	6.73** (March (=April) > June and July (=April))
Nutrient × month	3	ns

Note: Multiple comparison tests were conducted for significant ($p < 0.05$) effects of month with Tukey's test ($p < 0.05$). *, $p < 0.05$; **, $p < 0.005$; ns, not significant.

stream between high-light and low-light times of the year (Kruskal–Wallis, $\chi^2 = 13.71$, df = 3, $p < 0.005$; Tukey's multiple comparison, $p < 0.05$) (Fig. 5*d*). This difference was likely due to the unusually high biovolume measured in the reference stream in September 2001.

Growth rates

Nutrients had a significant positive effect on accrual rates of algal cells (Table 3; Fig. 6). Accrual rates were also significantly higher in March compared with June and July (Table 3), and the range in accrual rates in the treatment stream decreased by an order of magnitude from April to June and again by an order of magnitude from June to July (Fig. 6). However, differences between streams did not vary seasonally (nutrient by stream interaction was not significant) (Table 3).

Species composition

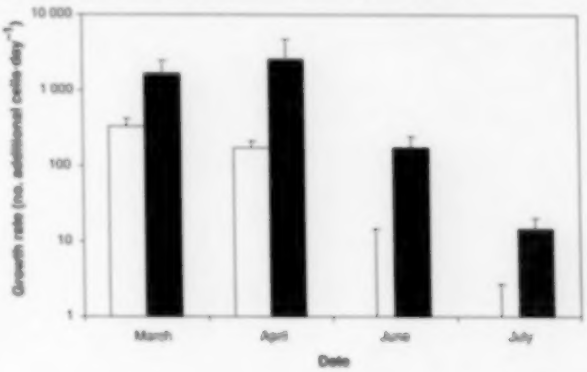
Diatom taxa were dominant (>98% of algal biovolume on average) in both the reference and treatment streams throughout the experiment (Table 4). Of the 35 taxa identified, most were rare, with five taxa making up nearly 95% of the biovolume in both streams (Table 5). None of these taxa showed significant differences in biovolume between the reference and treatment streams (RIA $p > 0.05$) (Fig. 7). By far, the dominant taxa in the streams were *Eunotia pectinalis* var. *minor* and *Meridion constrictum*, which exhibited somewhat predictable seasonal trends. Biovolume of *E. pectinalis* var. *minor* was low in the spring months and peaked in the late summer and fall (Fig. 7*a*), whereas biovolume of *M. constrictum* was greatest in spring and declined through the summer months (Fig. 7*b*). Although important contributors to total biovolume, *Eunotia pectinalis* var. *recta* (Fig. 7*c*) and *Gomphonema parvulum* (Fig. 7*d*) showed no strong seasonal patterns or treatment effects, except for an increase in *G. parvulum* in spring 2002. *Navicula tantula* showed a potentially delayed response to enrichment, increasing in relative biovolume from about 10% to nearly 50% in the treatment stream during the last two sampling dates (Fig. 7*e*).

Discussion

Effects of nutrient addition on stream periphyton

Our study showed how different aspects of the periphyton responded differently to nutrient enrichment, which may be useful in predicting chronic nutrient enrichment effects in similar headwater streams. Overall, we observed increased

Fig. 6. Increase in cell density per day ± 1 SE (logarithmic scale) between week 2 and week 4 of the algal growth experiment during March, April, June, and July trials for the reference stream (open bars) and treatment stream (solid bars).



algal productivity that resulted in small increases in periphyton biomass and no detectable change in algal assemblage composition. Algal cellular growth rates were stimulated by nutrient addition, but the magnitude of the response was small under seasonally low light levels (<100 cells·cm^{-2}·day^{-1}), suggesting constraints resulting from light availability. Periphyton biomass increased by some measures (chlorophyll *a* and AFDM) but not others (algal biovolume) and was small compared with standing crops of other food resources (e.g., leaf detritus) in the study streams (e.g., 0.5 g AFDM periphyton·m^{-2} compared with ~300–500 g AFDM leaves·m^{-2}; K. Suberkropp, Biology Department, University of Alabama, Tuscaloosa, AL 35487, USA, unpublished data). Notably, even the highest biomass values that we observed were low compared with benthic stream periphyton biomass measurements worldwide (Feminella and Hawkins 1995, Biggs 1996; Dodds et al. 1998). Effects of nutrient enrichment on biomass as chlorophyll *a* and cellular growth rates peaked during months with relatively higher irradiance; only biomass measured as AFDM, which includes heterotrophic components of periphyton, was consistently positively affected by nutrient enrichment. Short-term experimental studies have shown that a transient response to nutrient enrichment can be quite high in the absence of grazers and under high-light conditions (e.g., Rosemond 1993; Hillebrand 2002), whereas such responses are small when top-down consumption or other limiting factors also constrain algal biomass (e.g., Lowe et al. 1986; Peterson et al. 1993; Rosemond et al. 1993). Results from our long-term study in which periphyton biomass and taxonomic response was subdued or apparently controlled by other factors are consistent with such studies.

The size of effect that we observed on variables that responded to enrichment can be put into context of other responses of periphyton to environmental variation. For example, Feminella and Hawkins (1995) provided data from a wide range of stream systems examining grazing impacts on periphyton. For experiments that ran >4 weeks, chlorophyll a was ~40 mg·m^{-2} under grazed conditions and ~100 mg·m^{-2} under ungrazed conditions. In terms of defining nutrient-driven trophic state, Dodds et al. (1998) sug-

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Table 4. Average cell biovolume ($\mu m^3 \cdot cm^{-2}$) and standard deviations (SDs) of all cells and algal divisions for reference and treatment streams during the pretreatment year and both years of nutrient enrichment.

Site	Total	Diatoms	Chrysophyta	Chlorophyta	Cyanobacteria
Pre-enrichment					
Reference					
Mean	1.35×10^6	1.3×10^6	21 259	1 004	116
SD (n = 30)	0.72×10^6	0.72×10^6	44 721	2 460	285
Treatment					
Mean	1.90×10^6	1.89×10^6	13 632	0	0
SD (n = 30)	1.44×10^6	1.45×10^6	21 201	1	1
Post-enrichment year 1					
Reference					
Mean	1.49×10^6	1.45×10^6	28 547	423	252
SD (n = 29)	1.10×10^6	1.12×10^6	39 317	1 037	617
Treatment					
Mean	2.71×10^6	2.70×10^6	427	4 721	15
SD (n = 29)	1.00×10^6	1.00×10^6	744	11 565	37
Post-enrichment year 2					
Reference					
Mean	3.22×10^6	3.18×10^6	33 631	1 865	550
SD (n = 29)	4.42×10^6	4.43×10^6	46 535	2 515	953
Treatment					
Mean	1.65×10^6	1.65×10^6	403	4 714	23
SD (n = 30)	1.23×10^6	1.23×10^6	987	11 546	57

gested mean chlorophyll *a* of 20 $mg \cdot m^{-2}$ and maximum chlorophyll *a* of 60 $mg \cdot m^{-2}$ as the boundary between oligotrophic and mesotrophic systems. Our average chlorophyll during high-light months in the nutrient-enriched stream was ~10 $mg \cdot m^{-2}$, and there were only three monthly average measurements for which chlorophyll *a* was >15 $mg \cdot m^{-2}$. Biggs (1996) found a median of 1.7 mg chlorophyll $a \cdot m^{-2}$ for benthic periphyton in unenriched streams compared with a median of 21 mg chlorophyll $a \cdot m^{-2}$ for enriched streams. Our observed values in the treatment stream approached the median for Biggs' (1996) enriched values at certain times but were not sustained at that level. Our AFDM values were extremely low, even in the nutrient-enriched stream, compared with other studies. Feminella and Hawkins (1995) reported values of 5000 – 40 000 mg AFDM $\cdot m^{-2}$ from various studies and Biggs (1996) reported medians of 1500 mg AFDM $\cdot m^{-2}$ (unenriched) and 4800 mg AFDM $\cdot m^{-2}$ (enriched). Our values, even in the nutrient-enriched stream, were <1000 mg AFDM $\cdot m^{-2}$ (~250–550 in unenriched versus enriched stream on average). The lack of a strong biomass response in this study may have been due to control by other limiting factors that determine the chronically low standing crop of periphyton in these streams.

The duration of our experiment was likely long enough for potential changes in periphyton biomass and algal assemblages to occur. The other long-term whole-stream enrichment that we are aware of, conducted in the Kuparak River, represents 16 years of enrichment during the growing season (for ~45 days each year, total = 720 days) (Slavik et al. 2004). Because of the more moderate climate in our study area compared with the Alaskan tundra, our continuous enrichment represents 730 days for potential periphyton response. These conditions represent several to hundreds of generation times for algal populations, allowing for a maximum potential bottom-up response. Lengthening the time of our study would have likely only affected the potential for a nutrient response of periphyton to be constrained by consumption. Specifically, a longer time frame would allow greater potential for increased production of consumers to control periphyton response; larval life span of dominant potential herbivores in these streams ranges from 120 to 365 days (W. Cross, El Verde Field Station, HC-05 Box 8974, Rio Grande, Puerto Rico 00745, unpublished data).

Irradiance was likely an important factor in the expressed response of periphyton to nutrient enrichment. Seasonal variation in chlorophyll *a*, the amount of chlorophyll *a* per cell, and measures of algal cell growth pre- and post-canopy closure suggests that irradiance influenced all of these variables. The variation in nutrient concentrations in the enriched stream over the course of the enrichment did not likely have overriding effects on the seasonal responses of the above variables, as the peaks in nutrient levels measured in the stream did not coincide with peaks in chlorophyll measurements or growth rates. Seasonal variation in chlorophyll *a* appeared to be at least partly due to a trend of increased chlorophyll *a* produced per cellular unit rather than an increase in biovolume or cell densities. In previous studies, increased chlorophyll *a* per cell in microalgae occurred in response to increased nutrients (Rosen and Lowe 1984; Geider et al. 1993; Rosemond 1993). Interestingly, the nutrient-driven response of higher chlorophyll *a* per cell under high-light conditions that we observed is opposite to the predicted response (in the absence of enrichment) in which taxa with greater relative chlorophyll *a* per cell or a physiological shift to greater chlorophyll *a* per cell is observed in response to low-light environments (e.g., Rosemond 1993; Felip and Catalan 2000). Contributing to this pattern was that total biovolume was temporally vari-

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Table 5. Algal taxa encountered from periphyton samples on tiles.

	Reference	Treatment
Bacillariophyta		
Achnanthes deflexa	—	R
Achnanthes stewartii	R	R
Achnanthes subrostrata var. *appalachiana*	U	U
Achnanthidium minutissimum	R	R
Cymbella tumida	—	R
Diatoma hiemale var. *mesodon*	R	R
Encyonema minutum	R	R
Eunotia curvata	R	R
Eunotia exigua var. *exigua*	R	R
Eunotia pectinalis* var. *minor	A	A
Eunotia pectinalis* var. *recta	C	C
Fragilaria vaucheriae	R	R
Frustulia rhomboidies	R	R
Gomphonema acuminatum var. *pusillum*	R	R
Gomphonema gracile	R	R
Gomphonema parvulum	C	C
Melosira varians	—	R
Meridion constrictum	A	A
Navicula angusta	R	R
Navicula placenta	U	R
Navicula tantula	U	C
Nitzschia palea	R	R
Nizschia dissipata	R	R
Pinnularia mesogongyla	R	R
Pinnularia subcapitata var. *paucistriata*	—	R
Planothidium lanceolatum	R	R
Surirella angusta	R	R
Synedra minuscula	R	R
Synedra rumpens var. *meneghiniana*	R	R
Synedra ulna	R	R
Chrysophyta		
Unidentified stomatocysts	U	R
Chlorophyta		
Mougeotia sp.	R	R
Cyanobacteria		
Chamaesiphon sp.	R	R
Unidentified spheres	R	R
Unidentified filaments	R	R

Note: Categories based on average relative biovolume across all samples (% of relative biovolume): A, abundant (>20%); C, common (5–20%); U, uncommon (1–5%); R, rare (<1%). A long dash (—) means that the species was not detected in that stream. Combined species in bold accounted for 95% of the biovolume and were analyzed for responses to nutrient enrichment.

able, was not affected by enrichment, and showed a trend to be higher in low- versus high-light months, whereas chlorophyll *a* exhibited a response to nutrients in only high-light months. This suggests that physiological changes and the manufacturing of the nitrogen-containing chlorophyll molecule were limited by nutrient availability but were not consistently accompanied by increased algal biomass.

Stoichiometric changes in epilithon were also observed in which both the carbon to nitrogen and carbon to phosphorus ratios (8.7 to 4.6 and 1741 to 845, respectively) of epilithic scrapings in the treatment stream were greatly reduced compared with the reference stream (Cross et al. 2003). In the absence of substantial changes in periphyton assemblage composition, changes in stoichiometry may signal other physiological changes in periphyton in response to enrichment. However, since these nutrient concentrations were measured from the entire epilithic community, it cannot be determined if the source of change was from autotrophic, heterotrophic, or both components of the periphyton.

Our data indicate that heterotrophic compared with autotrophic components of periphyton biomass responded more consistently to nutrient enrichment. AFDM, which is a measure of periphyton biomass that likely includes heterotrophic components (e.g., fungi, bacteria, and trapped organic particles), increased in response to enrichment, but quantification of the strictly algal response, algal biovolume, did not. Heterotrophic microorganisms associated with leaves (Gulis and Suberkropp 2003) and wood (Gulis et al. 2004) increased dramatically in the treatment stream during this study, indicating that positive effects of nutrients on heterotrophs living on other substrates (e.g., tiles) would be likely. However, autotrophs may also have contributed to variation in AFDM via the production of extracellular organic material by algae, which could potentially increase in response to enrichment (Hoagland et al. 1993).

How much of a lack of algal response could be attributed to herbivore consumption versus light availability?

The seasonal pattern in algal growth rates and chlorophyll *a* was most likely the result of light availability in these headwater streams. The maximum response observed in chlorophyll *a* and growth rates, the only definitive responses to enrichment, occurred when light levels reaching the stream were high. Also, data from whole-stream metabolism measurements (P.J. Mulholland, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA, unpublished data) showed the highest rate of gross primary production (GPP) in both treatment and reference streams during April compared with other seasonal measurements, although overall, GPP was always very low (typically <3 mg $O_2 \cdot m^{-2} \cdot day^{-1}$ with one value of ~9 mg $O_2 \cdot m^{-2} \cdot day^{-1}$). Other studies have shown that benthic algal productivity can be primarily limited by light (Hill et al. 1995, 2001; Hill and Knight 1988) and that the importance of light, relative to other limiting factors, can change seasonally (Rosemond et al. 2000). Rates of photosynthesis generally reach a maximum at light levels between 100 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in forested streams (Hill et al. 1995). Instantaneous irradiance levels during spring and fall often reached or exceeded 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in both streams, indicating that light limitation was most likely less important during this time than during the summer when irradiance levels were <20 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Overall biomass of periphyton remained low in both streams despite increased productivity of algal assemblages as cellular growth rates in the treatment stream. Our measurements of cellular growth rates, which were greatest in magnitude prior to canopy closure (March and April), were

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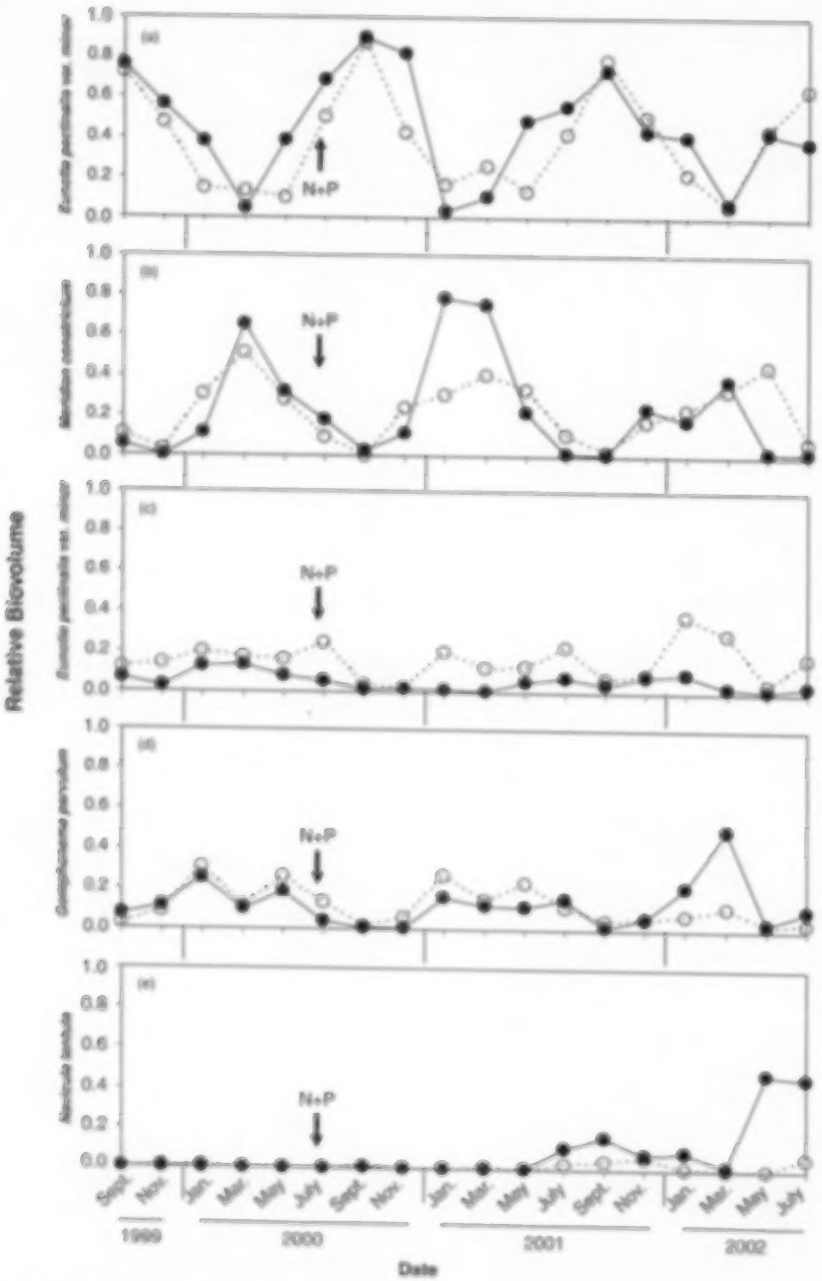
Fig. 7. Relative biovolume of the five most common species: (*a*) *Eunotia pectinalis* var. *minor*, (*b*) *Meridion constrictum*, (*c*) *Eunotia pectinalis* var. *recta*, (*d*) *Gomphonema parvulum*, and (*e*) *Navicula tantula* from the reference stream (open circles) and treatment stream (closed circles). Randomized intervention analysis (RIA) showed that differences between streams before and after enrichment were not significantly different.



made in the absence of potential macroinvertebrate grazers. It is possible that in situ consumption by invertebrate grazers precluded such increased productivity from resulting in higher algal biomass in the study stream. Specifically, despite observed nutrient-driven increases in algal growth rates that were fairly large in magnitude, rates of primary productivity in these streams are so low that invertebrate grazers can likely consume any accumulated biomass. As a potential indication of herbivores tracking and controlling algal resources in these streams, we examined whether invertebrate scraper biomass was similarly higher in higher light months, as we had observed with chlorophyll *a*. Over the time period

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of enrichment, mean monthly scraper biomass from rockface substrata was as follows: for June–October (n = 10 per stream), 23.1 mg·m^{-2} (3.4 SE) in the reference stream and 26.2 mg·m^{-2} (8.6 SE) in the treatment stream and for May–November (n = 14 per stream), 41.4 mg·m^{-2} (8.7 SE) in the reference stream and 91.2 mg·m^{-2} (29.6 SE) in the treatment stream (overall means based on n = 3 means from rockface substrate taken monthly; methods in Cross 2004). Scraper biomass was highest in the treatment stream during the high-light period but not significantly so and overall differences in scraper biomass between streams were not significant (Cross 2004). Carbon flow to scrapers was also on the order of GPP in these streams, suggesting that they could keep up with any excess primary production. Carbon production estimates translate to 0.2 g AFDM·m^{-2}·year^{-1} (based on GPP of 3 mg O_2·m^{-2}·day^{-1}), whereas carbon flow to scrapers was on the order of 0.3 and 0.5 g AFDM·m^{-2}·year^{-1} (reference versus treatment, year 1) and 0.2 and 0.4 g AFDM·m^{-2}·year^{-1} (reference versus treatment, year 2) (Cross 2004). These values suggest that overall primary production and flow of energy to scrapers was low, but an increased value in the treatment stream suggests some increased carbon going to scrapers under elevated nutrient conditions. Further, the increase in scrapers provides support for the possibility that autotrophic components of periphyton were under dual control by nutrients and light levels, since potential grazer response to nutrient addition was only during the spring months.

Limited assemblage composition response

There was remarkable constancy in the composition of the benthic algal assemblages in both study streams despite long-term changes in the availability of nutrients in the treatment stream. There were actually more pronounced seasonal effects in algal assemblages compared with nutrient effects, suggesting that light availability was driving the species distribution in this study. *Eunotia pectinalis* var. *minor* and *M. constrictum* seemed to trade off dominance in the community, with *E. pectinalis* var. *minor* making up most of the biovolume when light availability was seasonally low and *M. constrictum* dominating during periods of high light. *Eunotia pectinalis* var. *minor* also exhibited preference for low light levels in another deciduous forest stream study (Rosemond 1993), and *Meridion circulare*, a taxon related to *M. constrictum*, was shown to be stimulated by higher light levels (Rosemond et al. 2000), consistent with seasonal patterns observed in this study. However, *Meridion* has been shown to prefer colder temperatures (Patrick 1971; Lowe 1974), which may explain its peak during the colder months of the year. Toward the end of the study period, two taxa, *G. parvulum* and *N. tantula*, were potentially responding to enrichment, indicating that possibly the algal assemblage was beginning to change toward the end of the experiment. The increase in *G. parvulum* occurred during the high-light months and in another study at Coweeta was more common in a clearcut stream (Lowe et al. 1986), suggesting a preference for higher light levels. *Navicula tantula* has been described as preferring slightly enriched environments (Bahls 1993; Kentucky Division of Water 2002), which may explain its increase during the last two sampling dates.

The species pool found on tiles in these headwater streams was largely limited to diatoms, with little presence of chlorophytes or cyanobacteria. Whatever factors restrict taxonomic distribution in our samples may have ultimately constrained any response that we observed of benthic algae to nutrient enrichment. Chlorophytes might be expected more during seasonal periods of high light, as was observed in a nearby stream at Coweeta that had been logged (Lowe et al. 1986). Although no seasonal response of chlorophytes was seen, biovolume was slightly higher in the treatment stream with enrichment, indicating a potential response to nutrients. However, chlorophytes were too rare to submit to statistical analysis. Cyanobacteria have been known to outcompete diatoms in low-light situations (Stevenson et al. 1985) and could be expected to thrive during nutrient enrichment, but cyanobacteria were rarely encountered in our tile samples.

An analysis of algal communities from different substrata (moss, liverwort, and bedrock) from approximately the last two sampling dates of this study indicates that communities from tiles were largely representative of algal communities on natural substrates (Greenwood 2004). Bryophytes were important primary producers in these streams but showed no biomass response to nutrient enrichment (Greenwood 2004). About 80% of the taxa found on bedrock were also found on tiles (the other 20% dominated by cyanobacterial filaments), but dominant taxa from tiles only comprised 5%–65% of the biovolume on bedrock. In addition, the differences between assemblages on moss, liverworts, or bedrock in treatment versus reference streams were tested and no consistent changes from enrichment were found (Greenwood 2004). These results suggest that regardless of substrata, response of the algal community to nutrient enrichment was minimal.

The species pool of algae in these streams was mostly limited by factors other than nutrients. Other studies have found very little change in algal species composition in response to nutrients under conditions of intense grazing (assemblage composition was driven by grazer resistance more than by response to nutrients (e.g., Hill et al. 1992; Rosemond et al. 1993). However, the dominant taxa in our study streams would not necessarily be characterized as a flora indicative of a heavily grazed system (e.g., some taxa are stalked and produce upright cells in contrast with dominance by prostrate adnate taxa that are grazer resistant; Steinman 1996). Physical or chemical characteristics (overall low irradiance levels, high turbulence, and softwater chemistry) of these streams may also have overriding controls on potential assemblage composition. Regardless of which other controlling factors were most important, subtle physiological changes such as increased chlorophyll *a* or nutrient content did not translate into enough differential activity among different algal species to result in any shifts in species composition.

Two years of continuous moderate nutrient enrichment resulted in neither shifts in algal assemblage composition nor sustained large effects on algal biomass. This limited response can probably be attributed to the low initial standing crop of periphyton, other factors controlling biomass and productivity, and to a limited taxonomic pool of potential responders to nutrient addition. In contrast with nutrient effects on primary producers, analyses of heterotrophic

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microbial response in these study streams has revealed dramatic effects of nutrient addition on bacteria and fungi associated with leaves and wood (Gulis and Suberkropp 2003; Gulis et al. 2004; K. Suberkropp, Biology Department, University of Alabama, Tuscaloosa, AL 35487, USA, unpublished data). Although we have evidence that shading and potentially invertebrate consumption contributed to the limited response of benthic algae, other factors that ultimately limited the algal species pool are unknown but are likely associated with the physiochemistry of undisturbed shaded headwater streams. Our data show that in such streams, if otherwise undisturbed, periphyton will respond very little to moderate levels of long-term nutrient enrichment.

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A potential model system for studying the genetics of domestication: behavioral variation among wild and domesticated strains of zebra danio (*Danio rerio*)

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Abstract: The process of domestication in fish is fundamentally important to conservation efforts because of the extensive use of hatcheries to mitigate population declines. Research into the genetic changes associated with the domestication process in many endangered species is impeded by a lack of genomic tools, long generation times, and large space requirements. The study of the genetics of fish domestication could therefore benefit from the introduction of a model system. In this paper, we document behavioral and growth rate differences observed between a domesticated laboratory strain of zebra danio (*Danio rerio*) and a strain newly introduced into the laboratory from its native habitat in India. Domesticated zebra danio showed a higher degree of surface orientation, a reduced startle response, and higher growth rate compared with wild zebra danio. Wild–domesticated interstrain hybrids were intermediate in phenotype for all traits. When strains were reared together, most interstrain behavioral differences were maintained, indicating a genetic basis underlying the interstrain phenotypic variation. Phenotypic differences observed in this study are consistent with the effects of domestication in other fish species, indicating that the zebra danio can be used as a model system for studying the genetics of the domestication process in fish.

Résumé : Le processus de domestication chez les poissons est d'importance fondamentale dans les efforts de conservation à cause de l'utilisation répandue des pisciculteures pour enrayer les déclins de population. La recherche des changements génétiques associés au processus de domestication chez plusieurs espèces menacées est enrayée par la carence d'outils génomiques, les longues durées des générations et les besoins considérables d'espace. Il serait donc avantageux pour l'étude de la génétique de la domestication des poissons de posséder un système modèle. Nous décrivons dans ce travail les différences de comportement et de taux de croissance observées entre une race domestiquée de laboratoire du zèbre (*Danio rerio*) et une race récemment introduite au laboratoire depuis son habitat naturel en Inde. Par comparaison aux zèbres sauvages, les zèbres domestiqués montrent un plus fort degré d'orientation en surface, une réaction mitigée de surprise et un taux de croissance plus élevé. Les hybrides entre les races sauvages et domestiquées ont un phénotype intermédiaire pour toutes les caractéristiques. Quand les races sont élevées ensemble, la plupart des différences de comportement entre les races persistent, ce qui indique que la variation phénotypique entre les races a une base génétique sous-jacente. Les différences phénotypiques observées dans notre étude correspondent aux effets de la domestication chez d'autres espèces de poissons, ce qui laisse croire que le zèbre peut être utilisé comme système modèle pour l'étude génétique du processus de domestication chez les poissons.

[Traduit par la Rédaction]

Introduction

Declines in habitat quality and other anthropogenic impacts have necessitated the use of captive rearing as a conservation tool for many fish species across North America. Although captive rearing in hatcheries can ultimately increase population size by reducing mortality rates, there is a risk these populations will over time adapt to the captive environment. The hatchery environment imposes drastically different selection pressures on fish populations, including changes in water quality, habitat complexity, diet, and predation risk. This alteration of the selective regime can over several generations lead to the evolution of a domesticated phenotype, undermining the primary goals of conservation efforts.

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[3]My friend and collaborator William Rowland passed away suddenly during the preparation of this manuscript. His kindness, enthusiasm, and intelligence will be sorely missed. — B.D. Robison.

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Even when the population of conservation concern is not subject to domestication directly, interaction with domesticated populations of fish can have a detrimental effect on fitness. These fitness effects can be manifested as ecological effects of supplementation (McMichael et al. 1997; Nickelson 2003) in which competing hatchery fish cause population declines in wild populations. In addition, negative fitness consequences can also result from genetic interactions such as hybridization between wild and domesticated fish (Waples 1991, 1994). An understanding of the genetic changes underlying the domestication process is therefore of fundamental importance to conservation of endangered and threatened fish populations.

In a broad sense, domestication can be defined as the process by which a population of animals becomes adapted to the captive environment through a combination of genetic changes over time (selection) and environmentally induced developmental changes (Price 1984). Domestication can have profound effects on the morphology, physiology, and life history of fishes. In the case of aquaculture species, there is often explicit artificial selection on desirable traits such as high growth rate (Gjedrem 2000). This selection not only changes growth rate but is correlated with other behavioral (Gregory and Wood 1999*a*, 1999*b*) and physiological changes (Handy et al. 1999). Even when there is no explicit program of artificial selection, the hatchery environment represents an entirely different fitness landscape relative to the native habitat of fish, often leading to inadvertent selection on a variety of traits.

A variety of behavioral changes related to the domestication process have been documented in fish species, with studies in the family Salmonidae being the most prevalent (but see Ruzzante and Doyle 1991, 1993; Ruzzante 1994). The absence of predation in the hatchery environment can ultimately lead to a decline in the fright response in several species of salmonids, including brown trout (*Salmo trutta*) (Johnsson et al. 1996; Alvarez and Nicieza 2003), Atlantic salmon (*Salmo salar*) (Einum and Fleming 1997; Johnsson et al. 2001), and masu salmon (*Oncorhynchus masou*) (Yamamoto and Reinhardt 2003). Domesticated fish can also exhibit changes in aggression levels (Ruzzante and Doyle 1993; Ruzzante 1994), but these changes depend on the availability and method of delivery of food (Ruzzante and Doyle 1991). Dominance hierarchies are also affected by domestication, although there seems to be no general pattern to the direction of change associated with domestication (Berejikian et al. 1997; Einum and Fleming 1997; Metcalfe et al. 2003). Other effects of domestication in fish include a decline in stress response (Lepage et al. 2000), an increase in feeding rate (Yamamoto and Reinhardt 2003), and a decline in the use of cover (Alvarez and Nicieza 2003). Surface orientation is also increased in domesticated fish (Reinhardt 2001; Lucas et al. 2004).

Despite the importance of domestication in the context of endangered and threatened fish conservation, little is known about the genetic changes underlying the domestication process in fish. This is primarily because the salmonids, the fish species in which domestication has been most extensively studied, have relatively long generation times and require large amounts of rearing space. These logistical drawbacks limit the potential scope of genetic studies. In addition, genomic resources for many species in which domestication is relevant are also somewhat limited, although this is changing rapidly (Thorgaard et al. 2002).

Examination of the genetics of domestication would benefit greatly from the introduction of a genetic model organism. The zebra danio (*Danio rerio*) is a genetically well-characterized model organism that has been used primarily for developmental biology. With a generation time of 3 months and space requirements that are far more forgiving than those of salmonids, the zebra danio is potentially well suited as a model organism for studying the genetics of domestication. In addition to its experimental tractability, there is a large set of genomic tools available, including the complete genome sequence. Despite its tractability and abundant genomic resources, the zebra danio remains underexploited by researchers in organismal fields such as ethology and evolutionary biology. A few studies have examined zebra danio behavior at its most basic level (Delaney et al. 2002; Mann et al. 2003; Turnell et al. 2003), but only one documents behavioral differences among strains (Wright et al. 2003).

There are many behavioral changes associated with domestication in salmonids, including changes in dominance, aggression, and use of cover. Not all of these behaviors may be relevant in the context of zebra danio domestication. However, in both the salmonid hatchery and the zebra danio laboratory environment, food delivery is always from the surface and there is a complete lack of predators. These factors suggest that among the many behavioral changes associated with fish domestication, zebra danio adapted to the laboratory environment might exhibit changes in surface orientation and startle or fear response similar to those observed in salmonids.

The purpose of this study was to test whether wild and domesticated zebra danio displayed behavioral differences consistent with domestication effects in other fish. We hypothesized that (*i*) domesticated zebra danio would have a higher degree of surface affinity than wild zebra danio, (*ii*) domesticated zebra danio would have a reduced startle response relative to wild zebra danio, and (*iii*) these behaviors would have a genetic basis. In addition, we predicted that (*iv*) zebra danio adapted to the laboratory environment would exhibit faster growth rates than their wild counterparts. Evidence of domestication in zebra danio would indicate their suitability as a model for the study of the genetic changes underlying the domestication process.

Methods

Strains

Behavior and growth rate of wild, domesticated, and wild–domesticated hybrid strains of zebra danio were compared. The Nadia strain was collected from its native habitat in India (see www.zfin.org for details) and brought to the University of Oregon zebra danio facility (Eugene, Oregon). A subsample of the wild-caught individuals was bred to establish the Nadia strain. The fish used in this study were four generations removed from the wild-caught individuals and are considered a wild strain for the purposes of this study. While it is possible that domestication selection has resulted in a phenotypic change from the wild-caught individuals, the surface orientation and antipredator behavior of

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Table 1. Behaviors scored during prestimulus and poststimulus trials.

Behavior	Description
A	Fish moves into quadrant A, the upper left quadrant of the tank
B	Fish moves into quadrant B, the upper right quadrant of the tank
X	Fish moves into quadrant X, the lower left quadrant of the tank
Y	Fish moves into quadrant Y, the lower right quadrant of the tank
Touch	Fish touches the surface of the water
Freeze	Fish lies motionless on the bottom for more than 3 s

Table 2. Behavioral indices describing surface orientation and stimulus response.

Behavioral index	Description
Surface touches	Number of surface touches in the minute prior to stimulus
Surface affinity	Proportion of prestimulus time spent in quadrant A or B
Surface visits	(Number of visits to quadrant A or B)/activity level
Activity level	Total number of quadrants visited
Stimulus affinity	Proportion of time spent in quadrant A or X
Stimulus visits	(Number of visits to quadrant A or B)/activity level

the fourth generation fish were qualitatively identical to those observed in the wild-caught population (B. Robison, personal observations).

The TM1 strain was created from zebra danio obtained from a pet store in 1986 and were subsequently housed in the laboratory for at least 24 generations. This strain has a green fluorescent protein transgene that is constituitively expressed via a beta actin promoter (Gibbs and Schmale 2000). The expression of green fluorescent protein in this fish is detectable as a visible green emission under ultraviolet light, and this was used as a marker of genotype in the mixed tank experiments described below. There is no indication that TM1 fish that lack the trans-gene behave differently than TM1 fish with the trans-gene (B.D. Robison, personal observation).

All strains, including interstrain hybrids, were generated by mass matings of each strain. All fish in each experiment were of the same age and experienced the same rearing environment throughout life.

Behavioral assays

At 3 weeks of age, the fish were sorted into three tanks containing 10 TM1 fish each, four tanks containing 10 Nadia fish each, and four tanks containing 10 hybrids each. These tanks will be hereafter referred to as "pure" tanks. There were also four tanks each containing five TM1 fish and five Nadia fish, hereafter referred to as "mixed" tanks. An unexpectedly high mortality rate in the zebra danio colony during the experiment necessitated a second experiment using tanks of mixed genotypes. In this case, additional fish were generated from mass matings as before and at 3 weeks assigned one of six tanks each containing four Nadia and four TM1 fish. This second experiment is referred to as experiment B. In all experiments, some mortality occurred between the larval stage and the time of the behavioral assays. A total of 162 fish were assayed in both experiments. In experiment A, the pure tanks yielded 26 TM1, 37 hybrids, and 31 Nadias, while the mixed tanks yielded 19 TM1 and 11 Nadias. In experiment B, 23 TM1 and 15 Nadia fish were assayed.

At 3 months of age, the fish were assayed for behavioral variation by filming behavior and behavioral response to stimulus. A 40-L glass aquarium was placed in a 90 cm × 90 cm × 110 cm blind and filled to 45 cm with water from the zebra danio colony. The bottom of the tank was covered with gravel to minimize reflection. Grids were drawn at the vertical and horizontal midlines of the tank, dividing it into four quadrants. Each day of filming, tanks were randomly selected from the experiment for examination. Individual fish randomly selected from the chosen tanks were placed in the aquarium and allowed to acclimate for 20 min. At that point, a circular white disk (hereafter referred to as the stimulus) 10 cm in diameter was lowered beside the tank (2 cm from the side of the tank and 10 cm above the bottom) so that it was visible to the fish in the tank. The entire procedure was filmed, including the acclimation period.

The fish were then removed from the test arena and anesthetized with MS 222 (tricaine methanesulfonate). A digital photograph was then taken for length determination, the fish were weighed to the nearest 0.1 g, and genotype was determined (in the case of mixed tanks) by testing for green fluorescent protein expression with an ultraviolet light source (Sylvania, Danvers, Massachusetts).

Scoring

Behaviors were scored from videotape without knowledge of the genotype of the fish under examination. Video was monitored for the minute prior to and the minute after the stimulus being lowered into place. Behaviors were scored using IBM Via Voice software (IBM Corporation, White Plains, New York) and a headset microphone. At each instance of a behavior (listed in Table 1), the code word for that behavior was spoken. Via Voice then wrote that behavior to a word file and indicated the time of the behavior to the nearest second.

Once the raw data were scored, behavioral indices were calculated separately for the prestimulus and poststimulus phases (Table 2). Indices associated with surface orientation included the number of surface touches, the proportion of time spent in the upper half of the water column, and the proportion of total quadrant visits that were to the upper half of the tank. Indices associated with startle response to the stimulus included activity level, proportion of time spent in

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Table 3. *P* values from analysis of variance.

	Pure					
Measure	Genotype	N vs. T	T vs. H	N vs. H	Mix A genotype	Mix B genotype
Prestimulus						
Surface touches	0.0013	0.0007	0.0040	0.2940	0.2607	0.0049
Visits above midline	0.0047	0.0019	0.0398	0.0880	0.5648	0.1716
Time above midline	0.0223	0.0074	0.0893	0.1670	0.4970	0.3544
Quadrants visited	0.0670				0.6144	0.0308
Visits to stimulus	0.7919				0.7752	0.507
Time near stimulus	0.8220				0.1613	0.6378
Poststimulus						
Surface touches	0.1720				0.5615	0.0139
Visits above midline	0.7905				0.6963	0.1694
Time above midline	0.5777				0.7881	0.1676
Quadrants visited	0.0077	0.0032	0.1398	0.0423	0.224	0.0261
Visits to stimulus	0.0021	0.0012	0.0803	0.0216	0.3234	0.0040
Time near stimulus	0.0163	0.0066	0.1813	0.0702	0.5956	0.0039
Length	0.0001	0.0004	0.0106	0.0205	<0.0001	
Weight	0.0008	0.0002	0.0061	0.0169	0.0273	

Note: Behavioral data were ranked transformed prior to analysis. For pure tanks, results of pairwise comparisons of genotypes (N, Nadia; T, TM1; H, hybrid) are reported only with a significant genotype main effect. Mix A and mix B refer to the genotype main effect of experiments A and B, respectively.

the half of the tank near the stimulus, and proportion of total visits that were to the quadrants near the stimulus.

Data analysis

Behavioral data were not normally distributed and were therefore rank transformed prior to analysis (Lucas et al. 2004). For both pure and mixed tanks, we performed a priori tests of our hypotheses that domesticated fish would be more oriented toward the surface of the water, would be more likely to approach a novel stimulus, and would grow faster than wild fish. In the pure tanks, ranked behavioral data were analyzed using a hierarchical mixed-model analysis of variance, nesting tank within genotype. Tank was considered a random effect, and the main effect of genotype was tested using the Proc GLM random command within the SAS analysis package (SAS Institute Inc., Cary, North Carolina). The significance of individual mean differences was determined using the LSMEANS option from Proc GLM.

In the mixed tanks, ranked behavioral data were analyzed using a factorial mixed model analysis of variance with genotype as a fixed effect and tank and the tank by genotype interaction as random effects. Significance tests of main effects were conducted using the Proc GLM random statement as described above.

Results

Surface orientation

Pure tanks

Prior to presentation of the stimulus, significant effects of genotype were observed for surface touches per minute, proportion of visits above the midline of the tank, and proportion of time spent above the midline of the tank (Table 3). In all cases, Nadia fish were less oriented towards the surface of the water than the TM1 fish, and the hybrids were intermediate (Fig. 1). Pairwise multiple comparisons ($\alpha = 0.05$) indicated that the Nadia genotype was significantly different from the TM1 genotype for all measures but not significantly different from the hybrids. Hybrids also had significantly lower surface touches per minute and proportion of surface visits than the TM1 fish (Table 3). After presentation of the stimulus, the effect of genotype on measures of surface orientation was insignificant.

Mixed tanks

The same trends in mean values of surface orientation measures were observed in the mixed tanks as those seen in the pure tanks; Nadia fish tended to be less oriented toward the surface than TM1 fish (Fig. 1). However, no effects of genotype on surface orientation measures were observed in experiment A using mixed-genotype tanks (Table 3). When the mixed tank experiment was repeated with a larger sample size (experiment B), the same trends in surface orientation were observed (Nadia fish less oriented toward the surface). The differences among genotypes in the proportion of visits to the top of the water column and the proportion of time spent in the top half of the water column were not significant. However, there was a significant difference between the genotypes in the number of surface touches (Table 3). This difference in number of surface touches was maintained even after the stimulus was presented. With the exception of the number of surface touches in experiment B, after stimulus presentation, there were no significant differences among genotypes in surface orientation measures (Table 3).

Startle response and activity level

Pure tanks

Prior to presentation of the stimulus, there were no significant differences in proportion of visits to the stimulus side of the tank, the proportion of time spent there, or the overall

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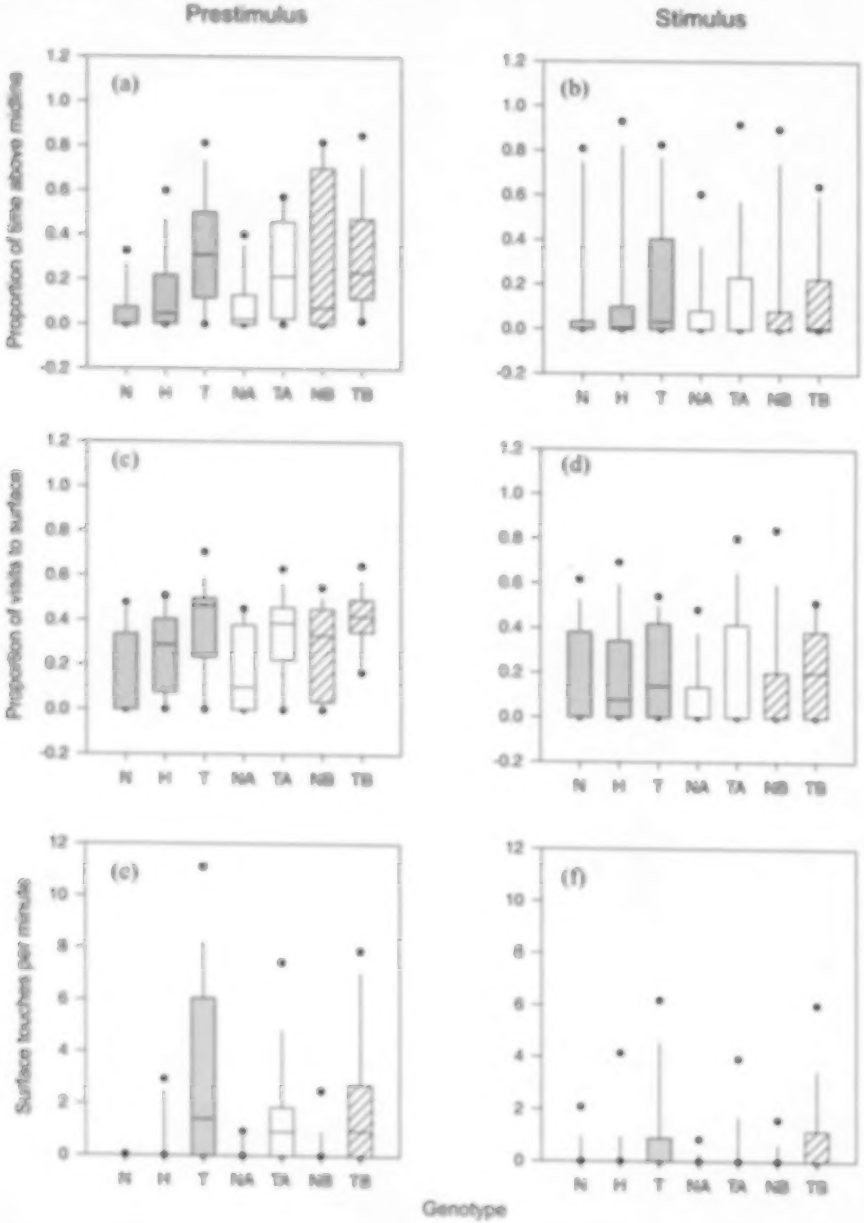
Fig. 1. Box plots of behavioral indices of surface orientation for wild (Nadia) (N), hybrid (H), and domesticated (TM1) (T) zebra danio (*Danio rerio*) housed in single-genotype tanks (shaded bars) or mixed-genotype tanks from experiment A (NA and TA) (open bars) and experiment B (NB and TB) (hatched bars). Box boundaries indicate the 25th and 75th percentiles and the line within the box indicates the median. The 10th and 90th percentiles are denoted by whiskers and dots indicate the 5th and 95th percentiles. The significance of statistical tests for each comparison is shown in Table 3.



activity level of the fish (Table 3). Subsequent to presentation of the stimulus, there were highly significant variations among genotypes in all three measures. The Nadia fish were less prone to visit the stimulus, spent a lower proportion of time near it, and showed a lower level of activity than the TM1 fish (Fig. 2). Pairwise multiple comparisons indicated that the Nadia genotype was significantly different from the TM1 genotype for all three measures. The Nadia genotype was also significantly different from the hybrids for both activity level and visits to the stimulus (Table 3).

Mixed tanks

The mixed tanks in experiment A showed no effect of genotype on any of the behavioral measures associated with

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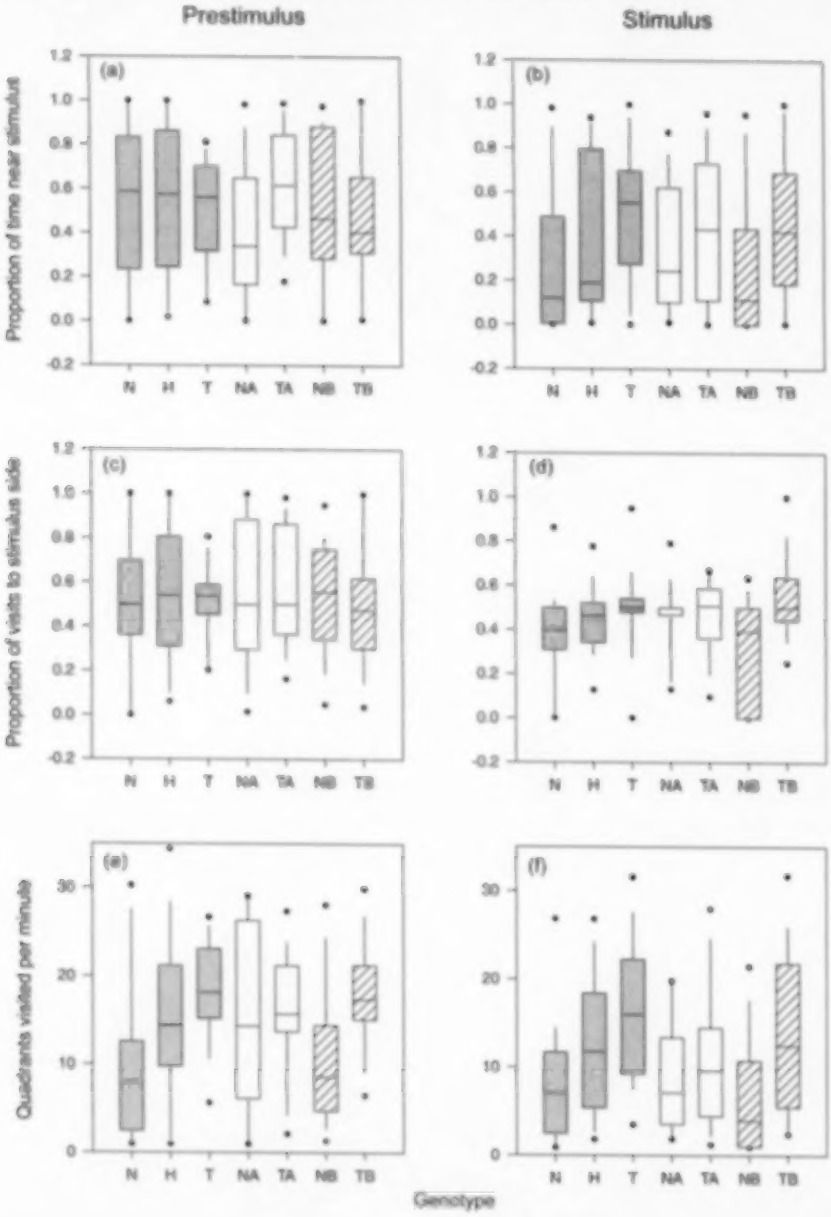
Fig. 2. Box plots of behavioral indices associated with response to a startle stimulus for wild (Nadia) (N), hybrid (H), and domesticated (TM1) (T) zebra danio (*Danio rerio*) housed in single-genotype tanks (shaded bars) or mixed-genotype tanks from experiment A (NA and TA) (open bars) and experiment B (NB and TB) (hatched bars). Box boundaries indicate the 25th and 75th percentiles and the line within the box indicates the median. The 10th and 90th percentiles are denoted by whiskers and dots indicate the 5th and 95th percentiles. The significance of statistical tests for each comparison is shown in Table 3.



startle response, both prior to and after presentation of the stimulus. When the experiment was repeated with a larger sample size (experiment B), there were significant differences between the Nadia and TM1 genotypes for activity level, visits to the stimulus, and proportion of time spent near the stimulus (Table 3) after the stimulus was presented.

Growth rate

TM1 fish were significantly larger in terms of weight and length than the Nadia fish. Hybrids were intermediate for both traits (Table 3; Fig. 3). In the mixed tanks, the difference between the Nadia and TM1 genotypes was greater than that observed in the pure tanks.

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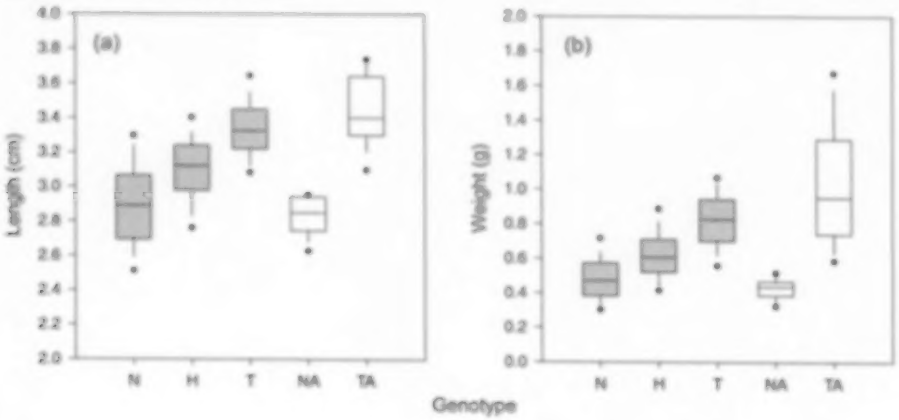
Fig. 3. Box plots of body size (length and weight) of wild (Nadia) (N), hybrid (H), and domesticated (TM1) (T) zebra danio (*Danio rerio*) housed in single-genotype tanks (shaded bars) or mixed-genotype tanks from experiment A (NA and TA) (open bars). Body size data were not taken in experiment B. Box boundaries indicate the 25th and 75th percentiles and the line within the box indicates the median. The 10th and 90th percentiles are denoted by whiskers and dots indicate the 5th and 95th percentiles. The significance of statistical tests for each comparison is shown in Table 3.



Discussion

Wild and domesticated strains of zebra danio displayed marked differences in surface orientation prior to stimulus exposure. The wild strain (Nadia) showed less inclination to be near the surface of the water than the domesticated strain (TM1) for all indices of surface orientation. After stimulus exposure, no significant effect of genotype was observed. These observations were mirrored for startle response, in which the domesticated genotype displayed a significantly higher affinity for the stimulus than the wild genotype. Our observations of a decline in fright response and an increase in surface orientation of the domesticated genotype are consistent with observations in other fish species and support our hypotheses that domesticated zebra danio show behavioral phenotypes similar to those observed in other domesticated fish species.

There are two possible explanations for the variation in behavior that we have observed among strains of zebra danio. First, the differences in behavior may reflect the evolution of a domesticated phenotype in the TM1 strain. Second, the behavioral variation could be a reflection of preexisting variation among the strains and is independent of the domestication process. We suggest that the most likely explanation is that the Nadia fish reflect a wild behavioral phenotype relative to the domesticated phenotype of the TM1 fish. There are two lines of support for this assertion. First, the behavioral patterns observed in this study are consistent with observations in other fish species that suggest that domesticated fish have a reduced fright response (Johnsson et al. 1996; Alvarez and Nicieza 2003) and a higher degree of surface orientation (Reinhardt 2001; Lucas et al. 2004). Second, two other domesticated strains that we are currently observing in the laboratory (the AB strain and a strain from Scientific Hatcheries, Huntington, California) show a similar behavioral phenotype to the TM1 strain (B.D. Robison, unpublished data).

Strong directional selection on captive fish populations can produce noticeable behavioral changes in as few as three generations (Ruzzante and Doyle 1991). This raises the possibility that the process of domestication is rapid and in the four generations prior to the experiment, the Nadia strain has effectively become domesticated. Under this scenario, the behavioral variation among strains observed in this study would not be a result of the domestication process. Although we cannot rule this out, our personal observations during this period indicate that there has been little behavioral evolution in the Nadia strain since it was collected.

A reduction in the fright response appears to be a general phenomenon during the domestication of mammals, birds, and fish (Grandin 1998; Price 2002). Although the reduction in fright response in domesticated fish has been most extensively documented in salmonids (Einum and Fleming 1997; Alvarez and Nicieza 2003), it has also been observed in summer flounder (*Paralichthys dentatus*) (Kellison et al. 2000) and now zebra danio (present study). The reduced fright response in domesticated fish is presumably a result of relaxation of selection associated with the complete absence of predators in the captive environment (Price 2002; Waples 1991).

The increase in the surface orientation of domesticated fish indicated by our data has also been observed previously in other species (Reinhardt 2001; Lucas et al. 2004). Changes in surface orientation during fish domestication could have many causes. Two primary candidate causes are a change in the fright response (discussed above) and the fact that in most cases of fish domestication, food is delivered to the surface of the water. Fish with reduced fearfulness and a greater likelihood to occupy the upper portions of the water column would therefore have greater feeding opportunities. If this were the case, one would predict that domesticated strains would have a higher growth rate when in competition with wild strains.

We observed significant differences in growth rate among wild and domesticated zebra danio, and these differences were maintained when the two strains were reared together. This is not an unexpected observation, as previous studies of

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domestication in salmonids have shown that the domesticated genotype has a higher growth rate (Einum and Fleming 1997; McGinnity et al. 2003; Yamamoto and Reinhardt 2003). The primary difference between the salmonids and the zebra danio is that in many cases of salmonid domestication, there is explicit selection on higher growth rate. In the case of zebra danio, there is no direct selection for higher growth. Our results therefore suggest that there may be inadvertent selection on growth rate, perhaps occurring as a correlated response to selection on reproductive capacity or on behaviors such as surface orientation. The increased growth rate in the domesticated strain may be a reflection of an increase in feeding opportunities associated with a higher affinity for the surface or a result of more efficient conversion of food into body mass in the domesticated strain. Further experiments will be necessary to identify the causes of growth rate variation among strains.

Hybrids between wild and domesticated zebra danio showed intermediate phenotypes for all behavioral and growth indices. This is a rough indication of additive gene action, although additional crosses are required to confirm this (Lynch and Walsh 1998). Prior experiments using hybrids between wild and domesticated salmonids have also shown behavioral and morphological phenotypes for hybrids that were intermediate between the parental strains (Einum and Fleming 1997).

The behavioral results in the mixed tanks suggest a significant genetic component to the behavioral variation among strains. When sample sizes were high enough (experiment B), the wild fish were significantly less oriented toward the surface and showed increased fearfulness. Even when statistical power was lacking (experiment A), the median behavioral indices reflected the trends observed in the pure tanks. This is an important observation if zebra danio are to be used as a model system for studying the genetic changes underlying the process of domestication. If surface orientation and approach behavior could be learned in a mixed-strain context, it would increase the error variance of behavioral measurements in recombinant (F2 or backcross) populations that would be used for genetic analysis. Increases in error variance of phenotypic measurements can cause significant reductions in statistical power, especially in experiments designed to detect quantitative trait loci (Lynch and Walsh 1998).

Although we know little about the natural history, habitat, and ecology of the zebra danio, it represents a complementary model system to species of conservation concern that have more studied ecologies. Domesticated zebra danio show qualitatively similar behavioral patterns to other domesticated fish populations, and this behavioral variation appears to have a significant genetic basis. The short generation time, forgiving space requirements, and robust genetic toolset of the zebra danio make it an excellent system for identification of the genetic changes associated with domestication. Genetic experiments requiring multiple generations of breeding (such as selection experiments and quantitative trait locus identification) can be rapidly conducted in zebra danio, and the large sample sizes required for these experiments can be easily achieved. The availability of a complete genome sequence (http://www.sanger.ac.uk/Projects/D_rerio/), saturation mutagenesis screens (Draper et al. 2004), and a dense genetic map (Shimoda et al. 1999) will allow identification of the genetic targets of domestication selection in the near future. These can then be tested as candidate genes in other species (such as salmonids and many species of cyprinids in the southwestern United States) in which domestication is a conservation concern.

Our data support the idea that there are commonalities in the domesticated phenotype among fish species. In both salmonids and zebra danio, domesticated strains are more oriented toward the surface, are less fearful, and have higher growth rates. The presence of common domesticated phenotypes in different species suggests that zebra danio can potentially be used as a model system for studying the genetics of domestication, which is an important phenomenon in fish conservation. In addition, the ideas presented here raise the question of whether the domesticated phenotype common to multiple fish species is a reflection of selection on similar physiological pathways or whether domestication is a case of convergent evolution. Incorporation of the zebra danio into studies of fish domestication is a first step towards addressing questions of this nature.

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Detecting p53 family proteins in haemocytic leukemia cells of *Mytilus edulis* from Pictou Harbour, Nova Scotia, Canada

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Abstract: Evaluating patterns of expression of p53-related proteins in cells is a novel approach in defining environmentally linked diseases. We have examined the induction of haemocytic leukemia in *Mytilus edulis* by municipal and industrial contaminants in Pictou Harbour, Nova Scotia, Canada. We used a murine monoclonal antibody, 1E10, as a diagnostic reagent to detect leukemic cells. We first characterized the reactivity of 1E10 with both normal and leukemic *Mytilus* haemocytes by confocal microscopy. We then compared p53 gene family expression (p53, p63–p73, and p97) in normal versus leukemic haemocytes using a panel of monoclonal and polyclonal antibodies to p53 family proteins. The immunochemical data demonstrate that haemocytic leukemia cells of *M. edulis* differentially express p63–p73 and p97–p120 proteins. We subsequently used 1E10 to diagnose haemocytic leukemia in 500 *M. edulis* previously deployed 6 months earlier in Pictou Harbour. In the field, *Mytilus* caged near untreated municipal wastewater and bleached kraft pulpmill effluents have a significantly greater chance of developing haemocytic leukemia than do mussels exposed to reference sites.

Résumé : L'évaluation des patterns d'expression des protéines de type p53 dans les cellules est une méthodologie nouvelle pour caractériser les maladies liées à l'environnement. Nous avons suivi l'induction de leucémie hémocytique chez *Mytilus edulis* par des contaminants municipaux et industriels dans le port de Pictou, Nouvelle-Écosse, Canada. Nous avons utilisé un anticorps monoclonal de souris, 1E10, comme réactif diagnostique pour déceler les cellules leucémiques. Nous avons d'abord caractérisé la réactivité de 1E10 avec des hémocytes normaux et leucémiques de *Mytilus* par microscopie confocale. Nous avons ensuite comparé l'expression des gènes de la famille p53 (p53, p63–p73 et p97) dans les hémocytes normaux par comparaison aux hémocytes leucémiques au moyen d'une gamme d'anticorps monoclonaux et polyclonaux dirigés contre les protéines de la famille p53. Les données immunochimiques démontrent que les cellules affectées de leucémie hémocytique de *M. edulis* ont une expression différente des protéines p63–p73 et p97–p120. Nous avons ensuite utilisé 1E10 pour déceler la leucémie hémocytique chez 500 *M. edulis* placés six mois auparavant dans le port de Pictou, Nouvelle-Écosse. En nature, les *Mytilus* encagés près des eaux municipales de rejet non traitées et les effluents de papetières à procédé kraft ont une probabilité significativement plus grande de développer une leucémie hémocytique que les moules exposées à des sites témoins.

[Traduit par la Rédaction]

Introduction

Haemocytic leukemia is a fatal disease that occurs in many molluscan species. The disease in molluscs was first diagnosed as a haematopoietic neoplasm Farley (1969). More recently, Miosky et al. (1989) and Kelley et al. (2001) have redefined haematopoietic neoplasm in *Mya arenaria* as a leukemia based on the overall pathogenesis of the disease. Yet, intense controversy remains among pathologists concerning the precise diagnosis of this disease. In clams, the terminology was reviewed by McGladdery and Bower (1999). Most recently, the Registry of Tumors in Lower Animals is entering cases diagnosed as leukemia as Leukemia:Systemic. New suggestions have included using disseminated neoplasia or haemocytic leukemia to indicate the putative cell of origin, the haemocyte. Based on our experience with monoclonal antibodies (Reinisch et al. 1983; Miosky et al. 1989), we have modified our use to include the word haemocytic.

Haemocytic leukemia has been reported in *Mytilus* subspecies along the northwest coast of the United States (Mix 1983; Elston et al. 1988; Krishnakumar et al. 1999), the United Kingdom (Green and Alderman 1983), Denmark

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(Rasmussen 1996), Spain (Alonso et al. 2001), Chile (Camplans et al. 1998), Italy (Zizzo et al. 1991), and southern British Columbia (Wong et al. 1984; Bower 1989). Haemocytic leukemia outbreaks in *Mya* have been documented in Chesapeake Bay (Farley et al. 1986), New Bedford Harbour (Reinisch et al. 1984), Long Island Sound (Brousseau 1987), and Prince Edward Island (McGladdery et al. 2001). In Prince Edward Island, for example, areas with haemocytic leukemia are subject to high agricultural runoff and (or) organochlorine wastes. Despite these many examples, the cause of molluscan haemocytic leukemia remains unresolved (Hillman 1993). Possible etiologies include the induction by environmental carcinogens and promoters and (or) infectious agents (Oprandy et al. 1981).

The most intensively studied molluscan haemocytic leukemia is that of *M. arenaria*, the soft-shell clam. Haemocytic leukemia in *Mya* is characterized by proliferating tumour cells in the haemolymph that have a high nuclear to cytoplasmic ratio. In end-stage disease, concentrations of haemocytic leukemia cells (HL) can reach 2×10^6 cells·mL haemolymph^{-1}. We developed a monoclonal antibody MAB 1E10 initially as a diagnostic reagent to detect haemocytic leukemia in the haemolymph of populations of *M. arenaria* (Reinisch et al. 1983). More recently, we extended these studies to investigate the structure of the protein recognized by 1E10 (Stephens et al. 2001). By primary structural analysis, we showed that the 1E10 antigen is a 252-kDa glycoprotein that shares homologies with spectrin–dystrophin and talin. These properties strongly suggest that the 1E10 structure is a transmembrane protein with potential cytoskeletal connections (Stephens et al. 2001).

Stephens et al. (2001) and Kelley et al. (2001) further characterized haemocytic leukemia cells from *M. arenaria* using monoclonal and polyclonal antibodies to proteins of the p53 gene family. p53 genes (53, 63–73, 97, and 120) regulate cell division and differentiation and are extremely important in pathways leading to either cell death or malignancy. While normal haemocytes of *Mya* express p53 and p97 but not p73, haemocytic leukemia cells express p53 and p73 but become p97 negative. We also identified an intermediate or transitional haemocyte in *Mya*, a cell that expresses the 2A4 antigen recognized by a murine monoclonal antibody (White et al. 1993).

Much less is known at either the molecular or cellular level about haemocytic leukemia in the blue mussel *Mytilus edulis*. Haemocytic leukemia in *Mytilus* was studied by Noel et al. (1994) who developed a panel of monoclonal antibodies that specifically recognize leukemic *Mytilus* haemocytes. These reagents, as well as molecular probes, provide far more sensitivity than does classic histopathology in detecting low numbers of abnormal cells or genes. The application of new technologies allows a far more accurate determination of marine and, ultimately, ecosystem health.

In this paper, we first documented that the murine monoclonal antibody 1E10 was specifically reactive with *Mytilus* haemocytic leukemia cells. Next, we compared the reactivity of *Mytilus* and *Mya* haemocytes on immunoblots using antibodies to different p53 gene family products. We also quantified protein differences in *Mytilus* by SDS–PAGE, finding the same distinctions as in *Mya*. We then used 1E10 to examine the haemolymph of *Mytilus* that had been deployed 6 months previously at 14 sites in and around Pictou Harbour, Nova Scotia, a site heavily contaminated with industrial and municipal wastes (Painter and Stewart 1992). The data suggest that haemocytic leukemia is potentially induced in Pictou Harbour by environmental contaminants but do not rule out a viral component.

Materials and methods

Haemolymph assessment

To determine if 1E10 could be used as a diagnostic reagent, we collected *M. edulis* from Pictou Harbour and analyzed them in 2001. The haemolymph was collected and screened by phase-contrast microscopy. The presumed leukemic cells were then fixed in 1% formalin, shipped to Woods Hole, Massachusetts, washed, and treated with a panel of monoclonal or polyclonal antibodies. The first set of analyses on cells from six *M. edulis* indicated that 1E10 was selectively reactive with a subset of haemocytes from Pictou Harbour. Based on those results, we designed this study and repeated these analyses with extensive controls.

In 2002, 1680 blue mussels were collected from a clean site, Malagash Bay, Nova Scotia, and caged at 14 different sites in industrialized Pictou Harbour. Malagash is a small harbour with no industrial input and is sparsely populated. Prior to the transfer, the mussels were tested by the molluscan health section of Fisheries and Oceans Canada and declared disease free. Each mussel was bled using a sterile 23-gauge needle inserted directly into the posterior adductor muscle sinus. One tenth of a millilitre of haemolymph was extracted into a dry 3-mL syringe and a preliminary diagnosis immediately performed using phase-contrast microscopy at 63× magnification. Haemocytic leukemia was initially assessed by cell morphology and by cell number using a haemocytometer. To confirm the diagnosis of haemocytic leukemia, we rebled mussels and fixed the haemolymph in modified Barker's formol calcium that contained 4% *v/v* formaldehyde, 2% *w/v* sodium chloride, and 1% *w/v* calcium acetate diluted in high-salt Tris-buffered saline (high salt). The resulting samples were stored in 2-mL Eppendorf tubes, numerically coded, and sent to Woods Hole where assays were performed along with presumed negative samples. Thus, every sample with a preliminary diagnosis of haemocytic leukemia was retested in Woods Hole along with negative samples using either controls or monoclonal antibody 1E10 to confirm the diagnosis.

Samples were subsequently spun three times for 5 min in high salt at 3000 rpm using an Eppendorf model 5415C microfuge (Eppendorf AG, Hamburg, Germany). Either high-salt solution, monoclonal antibody 1E10 (1:5), or normal mouse IgG was added to pelleted cells as previously described (Reinisch et al. 1983). The samples were then incubated on a rocker at 10 °C overnight, spun three times in high salt, and a 1:100 dilution of tetramethylrhodamine-conjugated (RITC) rabbit antimouse IgG (Molecular Probes, Eugene, Oregon) added to each sample. All antibodies were spun at 10 000 r·min^{-1} for 20 min prior to use to remove aggregates. The cells were then incubated an additional 2 h at 10 °C, washed three times, and evaluated using an Olympus BH2 microscope (Olympus Corporation, Tokyo, Japan) at 500× magnification using a rhodamine filter. Any positive

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samples were then visualized at an excitation wavelength of 488 nm using a Zeiss LSM 510 laser scanning confocal microscope (Zeiss, Oberkochen, Germany). In each analysis, normal IgG as well as high-salt solution were used to control for nonspecific binding of the secondary antibody or background signal.

Antibodies

Murine monoclonal antibody 1E10 that recognises *M. arenaria* haemocytic leukemia cells was developed as previously described (Reinisch et al. 1983). Murine monoclonal antibody 2A4 that recognizes *Mya* normal and intermediate haemocytes was developed as previously described (White et al. 1993). A rat monoclonal antibody to the beta subunit of tubulin (clone TUB 2.1) was obtained from ICN (Irvine, California). The universal p53 antibody Ab-1 (clone PAb421) was purchased from Calbiochem (La Jolla, California). An affinity-purified rabbit polyclonal peptide antibody to the *M. arenaria* (soft-shell clam) p53 family (p53–73) was the kind gift of Dr. Charles Walker (Department of Zoology, University of New Hampshire, Durham, NH 03824, USA). An affinity-purified rabbit polyclonal peptide antibody to the unique homology domain of p63–p73 (not present in p53) using the *Spisula solidissima* (surf clam) sequence (Cox et al. 2003) was prepared by New England Peptide, Inc. (Fitchburg, Massachusetts).

Sample preparation

Liquid nitrogen frozen pellets of haemocytes from individual mussels were mixed rapidly with 20 µL of 0.1 mol phenylmethylsulfonyl fluoride·L^{-1} in isopropanol and 0.4 mL of SDS sample buffer (2% SDS, 0.125 mol Tris–glycine·L^{-1}, pH 6.8, 1% 2-mercaptoethanol, 10% sucrose), sonicated with a microprobe at 10 W for 30 s (Sonicator model W-10), and boiled for >3 min. The samples were frozen at –20 °C, thawed, and spun at room temperature for 10 min at 14 000 r·min^{-1} (Eppendorf model 5415C) to remove insoluble debris. The resulting supernatants were removed and stored at –80 °C until use.

SDS–PAGE and immunoblot analyses

Samples were routinely analyzed on 1.5-mm-thick small-format conventional SDS – 8%T/2.5C (8% total acrylamide of which 2.5% is cross-linker) polyacrylamide gels using the method of Laemmli (1970). For greater resolution in the high molecular weight region, low cross-linked (8%T/1%C) polyacrylamide was used (Stephens et al. 2001). Gels were stained with Coomassie Brilliant Blue R-250 (Serva No. 35051) using the equilibrium method of Fairbanks et al. (1971). Immunoblotting to nitrocellulose was carried out by the renaturing method of Dunn (1985) followed by blocking with 1% bovine serum albumin (BSA) and 5% nonfat dry milk in Tris-buffered saline, a 1-h incubation with primary antibodies at dilutions specified in the text below, and detection with an alkaline phosphatase coupled secondary antibody and Western Blue stabilized substrate (both obtained from Promega Corporation, Madison, Wisconsin). Glycoproteins were detected on nitrocellulose blots with a glycoprotein detection kit from BioRad Laboratories (Hercules, California) (No. 170-64900). Video densitometry was used for quantification of stained gels and immunoblots as described in detail elsewhere (Stephens 1997 and references therein). Graphical analyses of densitometric data were performed with SigmaPlot 5.0 (SPSS, Inc., Chicago, Illinois) using its spreadsheet function to normalize band amount and position.

Study area

Pictou Harbour, located in northern Nova Scotia, is an important commercial estuary and has a history of environmental contamination dating back to the 1800s (Painter and Stewart 1992). These authors conducted an assessment of the environmental quality of Pictou Harbour and surrounding watershed (Painter and Stewart 1992), which evaluated all available information on the quality of waters, sediments, and biota and reported a variety of environmental problems. The following concerns for both Pictou Harbour and the East River were reported: high fecal coliform concentrations causing shellfish harvesting and beach closure, the presence of pollutants, such as metals and organic compounds including polycyclic aromatic hydrocarbons, polychlorinated biphenyls and furan, in the sediments, and dioxin and furan residues in shellfish and crustaceans from Pictou Harbour. Several studies have concentrated on contaminants in Pictou Harbour. For example, Dalziel et al. (1993) examined contamination in the sediment and water column and found slightly elevated heavy metal loads in the Pictou Harbour – Pictou Road sediments: 1–56 mg Cu·kg dry weight^{-1}, 0.01–0.85 mg Hg·kg dry weight^{-1}, 5–57 mg Pb·kg dry weight^{-1}, and 7–231 mg Zn·kg dry weight^{-1}. Prouse (1994) ranked Pictou Harbour the fourth most contaminated harbour among 14 examined in the Maritimes for polycyclic aromatic hydrocarbons levels in American lobster (*Homarus americanus*), and St-Jean et al. (1999) reported levels of tributyltin reaching 250 ng·g^{-1} in whole-mussel tissue. In addition, St-Jean et al. (2003) reported accumulations of several metals in mussels caged throughout Pictou Harbour: up to 31 µg Al·g wet weight^{-1}, 3.6 µg As·g wet weight^{-1}, 4.0 µg Cu·g wet weight^{-1}, 68 µg Fe·g wet weight^{-1}, 9.6 µg Mn·g wet weight^{-1}, and 26 µg Zn·g wet weight^{-1}. The sites from that study corresponded to sites PS1, PM-A, PM-B, LH, and LP from this study (see below).

Site selection and cage positions

The location of the various effluent types examined in this study are shown in Fig. 1. Along the East River, the frames at New Glasgow bridge were positioned approximately 100 m from the source point, which consist of a steel plant and buried city dump. The frames at a secondary-treated municipal wastewater site were positioned approximately 3 m from the source point (i.e., from the pipe outfall) of the secondary-treated municipal wastewater site. The frames at a coal-generated electrical powerplant were positioned approximately 300 m from the discharges of the effluent from the power plant. The frames at Mussel Point were positioned at the confluence of the East River and Pictou Harbour and assessed the cumulative contribution of the East River as well as the extent of the raw sewage plume.

In Pictou Harbour, frames were positioned in a gradient to assess the contribution of raw sewage (PS1, PS2, PS3, PS4, and PS5): there are two pipes discharging raw sewage from Pictou (population of 5000), one situated at PS2 (main dis-

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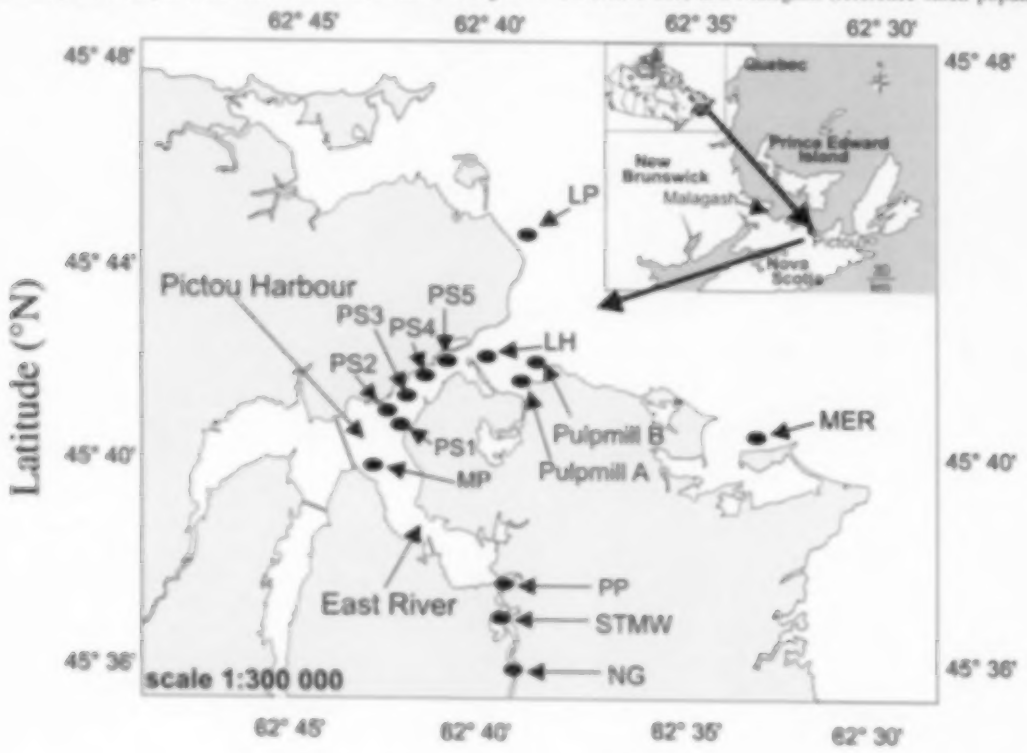
Fig. 1. Sampling sites around Pictou Harbour, Nova Scotia, Canada. NG, New Glasgow (steel plant and buried city dump); STMW, secondary-treated municipal wastewater; PP, power plant (coal-operated power generation); MP, Mussel Point; PS1–PS5, raw sewage discharged by the Town of Pictou (outfall on PS2 and PS3); LH, Light House; pulpmills A and B, both 500 and 1000 m from pulpmill discharge; LP, Logan Point (reference site); MER, Merigomish (reference site) and Malagash (reference stock population).



charge) and the second at PS3; thus, PS3 receives inputs from both outfalls. All of the PS frames were positioned in a 300-m interval gradient starting from PS2 and Mussel Point was positioned approximately 500 m from PS2.

For Pictou Road, the frames at Light House were positioned close to the narrow passage between Pictou Harbour and Pictou Road and monitored the extent of the Pictou Harbour contribution as well as the pulp mill effluent. Two frames were positioned in a gradient from the pulpmill effluent (pulpmills A and B). The frames were positioned 500 and 1000 m, respectively, from the discharge point of bleached kraft mill pulp and paper mill effluent into Pictou Road.

Mussels were also placed at two sites outside the harbour (LP and MER). LP is potentially under intermittent, or at least reduced, influence from Pictou Harbour, while MER is not under any harbour influence and was chosen to serve as a reference site.

All sites situated along Northumberland Strait, where the mussels originate, are designated pure populations of *M. edulis* with no *Mytilus trossulus* present (Mallet and Carver 1995). Morphometric measurement techniques developed by Mallet and Carver (1995) were repeated to confirm the identity of *M. edulis*.

In situ monitoring

In early March 2002, 1680 mussels measuring between 6 and 8 cm (adults) were collected from Malagash Bay. Upon arrival at the laboratory facilities in Pictou, the mussels were individually screened for size and loaded into socks (10 mussels per sock), each separated by cable ties. The socks were randomly divided among 28 frames (two frames per site). Each frame, made of ABS plumbing-grade tubing, held six socks for a total of 60 mussels per cage. The frames were deployed 2 m from the bottom in 4.5-m-deep waters in late March and retrieved for assessment in early October 2002, ensuring an exposure period of 6 months. Following retrieval, the mussels were transported to the laboratory and held before evaluation in a cooler containing water from the individual collection sites. Any dead or moribund mussels were discarded. A minimum of 28 mussels was assessed per site. In early July, the mussels spawned.

Statistical analysis

Owing to the nature of the data (assessments ranked in categories rather than continuous measures), we compared the levels of haemocytic leukemia at each site with the levels found at the reference site using the Kruskal–Wallis (KW) nonparametric analysis of variance test. The analysis was

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followed by a two-tailed nonparametric multiple comparison testing the rank mean of each site against the reference population (MER) independently (Zar 1984).

Results

Confocal microscopy

Haemolymph from four mussels from the Mussel Point and four mussels from the New Glasgow site were isolated for confocal analysis. Haemocytes from the Mussel Point site did not react with 1E10 (Figs. 2A.1 and 2B.1). In contrast, a majority of haemocytes from the New Glasgow site were specifically reactive (Figs. 2C.1 and 2D.1). These are typically large, rounded cells showing both plasma membrane and internal vesicular staining. Unstained cells were often irregular (Figs. 2C and 2D, arrows). One out of 70 Mussel Point cells stained with 1E10 versus 36 out of 68 New Glasgow cells. There was no reactivity above background when any cell population was incubated with either normal mouse IgG or high-salt solution as a first step.

We further evaluated haemocytic leukemia cell reactivity with the murine monoclonal 2A4, polyclonal Ab-1 (pan specific p53), and the rabbit homodomain antibody (p63–p73). As a control for background staining with the homodomain antibody, normal rabbit serum at the same protein concentration was used and the samples were processed with goat antirabbit RITC. Independent analyses showed that *Mytilus* haemocytic leukemia cells did not specifically react with 2A4, Ab-1, or the rabbit homodomain antibody to p63–p73. However, all but the first can detect antigen on immunoblots (below), suggesting specificity for denatured proteins.

Taken together, the data show that *Mytilus* haemocytic leukemia cells react specifically with murine monoclonal antibody (1E10) generated to *M. arenaria* haemocytic leukemia cells. In the next series of experiments, we analyze expression of p53 gene family members as well as structural proteins using Western blotting and quantitative SDS–PAGE.

Western blot analyses

Several pan-specific antibodies reveal key differences between normal and haemocytic leukemia cells in *Mytilus* haemocytes (Fig. 3) when analyzed at equal protein loadings (equivalent stained gels in Fig. 4). Tubulin is more prevalent by a factor of more than 3 in the presumably more rapidly dividing haemocytic leukemia cells. Similar ratio differences are seen with antibodies that recognize variants of the p53 gene family. A widely used antibody to p53 detects no 53-kDa protein in either cell type but does cross-react with a 120-kDa variant found almost exclusively in haemocytic leukemia cells. An antibody to the unique homology domain of the p63–p73 branch also shows a three- to four-fold greater amount of p63 in haemocytic leukemia cells. An antibody to a common region of the family also confirms the absence of significant amounts of p53 protein while detecting p63, p73, and a number of higher molecular weight variants, likely arising by posttranslational modifications (Cox et al. 2003). Although similar, this pattern is more complex than in *Mya*, where normal cells uniquely express a 97-kDa protein, while haemocytic leukemia cells exclusively express p73, with p53 remaining constant (Stephens et al. 2001). We provide a compilation of the reactivity of normal and haemocytic leukemia *M. edulis* and *Mya* arenaria haemocytes on immunoblots using both monoclonal and polyclonal antibodies (Table 1).

Protein analysis

We next compared the protein content and composition of haemocytes from *Mya* and *Mytilus*. Sixteen *M. edulis* haemocyte samples processed by conventional SDS–PAGE revealed a wide variation in total protein yield, ranging from <0.1 to >2 mg per individual (result not shown). The low-protein samples were very similar in polypeptide composition, as were three very high samples. Intermediate- and high-protein samples closely resembled the profile of *M. arenaria* haemocytic leukemia cells.

To conveniently compare protein composition qualitatively, we diluted the sample of highest protein content and ran it in parallel with normal haemocytes obtained from uncontaminated Cape Cod waters (Fig. 4). When loaded at equal actin content and analyzed on a low cross-linked SDS–PAGE system to better resolve high molecular weight proteins, characteristic differences become apparent by simple inspection of juxtaposed lane photographs. Normal cells uniquely contain a high molecular weight protein (N, ~300 kDa). Haemocytic leukemia cells contain material that barely penetrates the gel (HMW) and also contain a prominent protein of ~250 kDa (E), which runs as a doublet. Many additional lower molecular weight proteins further distinguish the two cell populations in this side-by-side comparison.

To refine the comparison and obtain quantitative information, we combined two additional samples, appropriately diluted them, and mixed them with an equivalent amount of normal cell extract for SDS–PAGE analysis (Fig. 5A). The characteristic proteins N and E, a second band (E′) now resolved in haemocytic leukemia cells from a band common to both, a large protein consistently diminished in haemocytic leukemia cells (A), and six additional proteins (asterisks) are noted. All of these have counterparts that distinguish normal from haemocytic leukemia cells in *M. arenaria* (Stephens et al. 2001).

The most obvious differences occur in the high molecular weight region (Fig. 5B) and several of these differences can be easily evaluated by densitometry after normalization (Fig. 5C). As was true in *Mya*, protein N is present only in normal cells, while protein E, the equivalent of the 252-kDa 1E10 antigen of *Mya*, is characteristic of haemocytic leukemia cells. The latter protein in *Mya* is often observed as an equimolar doublet, and therefore, E′ in *Mytilus* may correspond to the second *Mya* 1E10 band. Conversely, the 165 kDa band A is diminished, although not absent, in haemocytic leukemia cells, and consequently, it may be the equivalent of the 185-kDa 2A4 antigen in *Mya* normal cells. However, neither putative equivalent in *Mytilus*, E or A, was cross-reactive with the corresponding *Mya* antibody by immunoblotting and neither proved to be significantly glycoslyated (data not shown), a factor that, in addition to species affinity differences, may influence cross-reactivity or renaturation.

In situ monitoring

During the course of the exposure, the cage positioned at PS4 moved slightly outside the channel and thus outside the

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Fig. 2. Normal and leukemic haemocytes from *Mytilus edulis* collected from either Mussel Point (MP) or the buried city dump (NG) treated with murine monoclonal antibody 1E10 and analyzed with laser scanning confocal microscopy. The images were obtained using Karl Zeiss LSM 510 software. (A) Mussel No. 1 from MP; (B) mussel No. 2 from MP; (C) mussel No. 5 from NG; (D) mussel No. 8 from NG. Left panels: fluorescence image; right panels: Nomarski differential interference contrast image. Arrows in Figs. 2C and 2D indicate typical 1E10-negative cells. Scale bars = 5 µm.

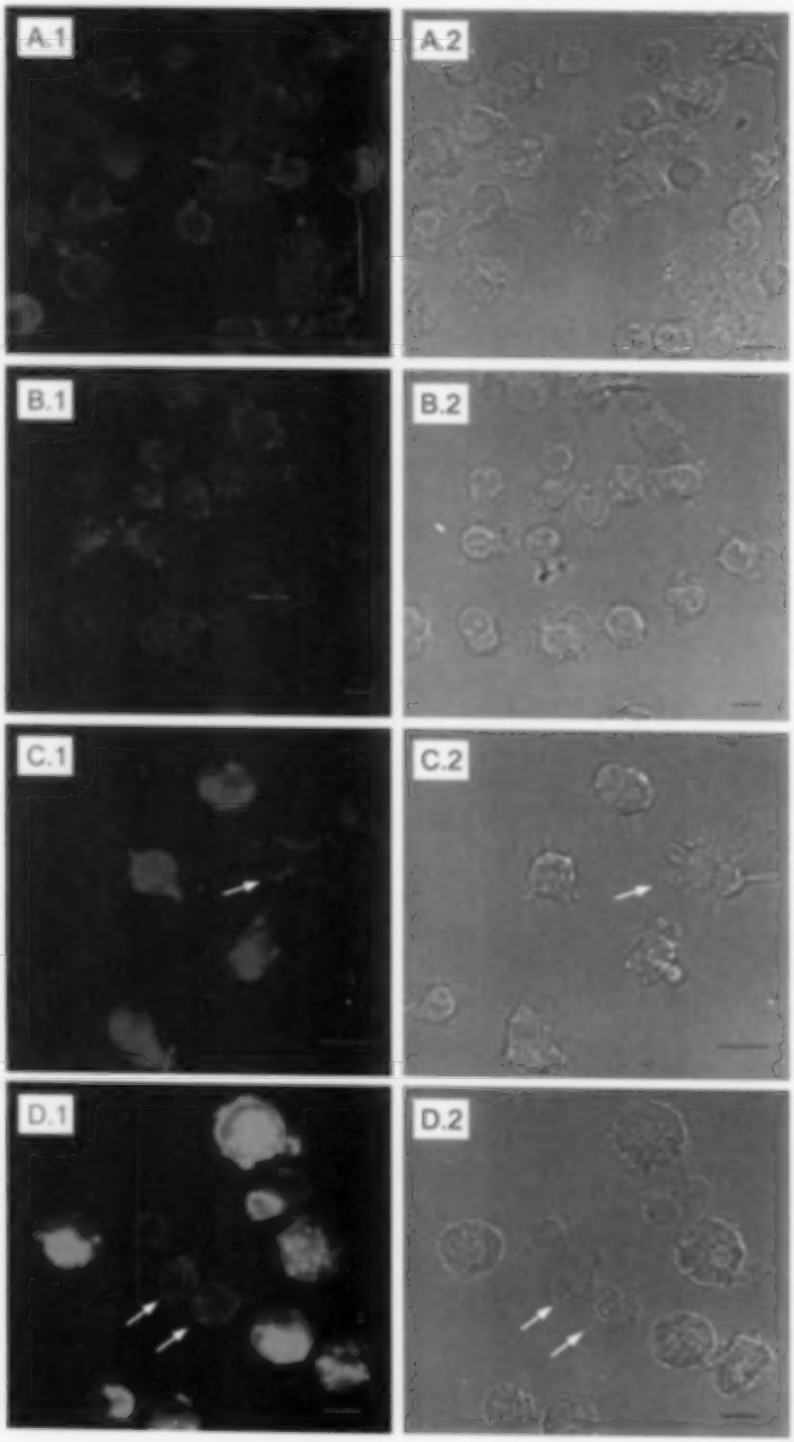

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Fig. 3. Comparative antibody cross-reactivity of *Mytilus* normal (N) and haemocytic leukemia (L) haemocytes at equivalent protein loading. Tubulin, mouse monoclonal TUB 2.1 (to rat beta chain; Gozes and Barnstable 1982) used at 1:1000 dilution; p53, mouse monoclonal Ab-1 (to human; Harlow et al. 1981) used at 1:100; p53–p73, rabbit polyclonal to a 23aa *Mya* peptide sequence from Kelley et al. (2001) used at 1:1000; p63–p73, rabbit polyclonal to a Spisula p63–p73 homology domain defined by Jessen-Eller et al. (2002) and Cox et al. (2003) used at 1:200. MW, molecular weight standards; asterisks, actin "ghosts" detected by p53–p73 antibody verifying equivalent protein loading.

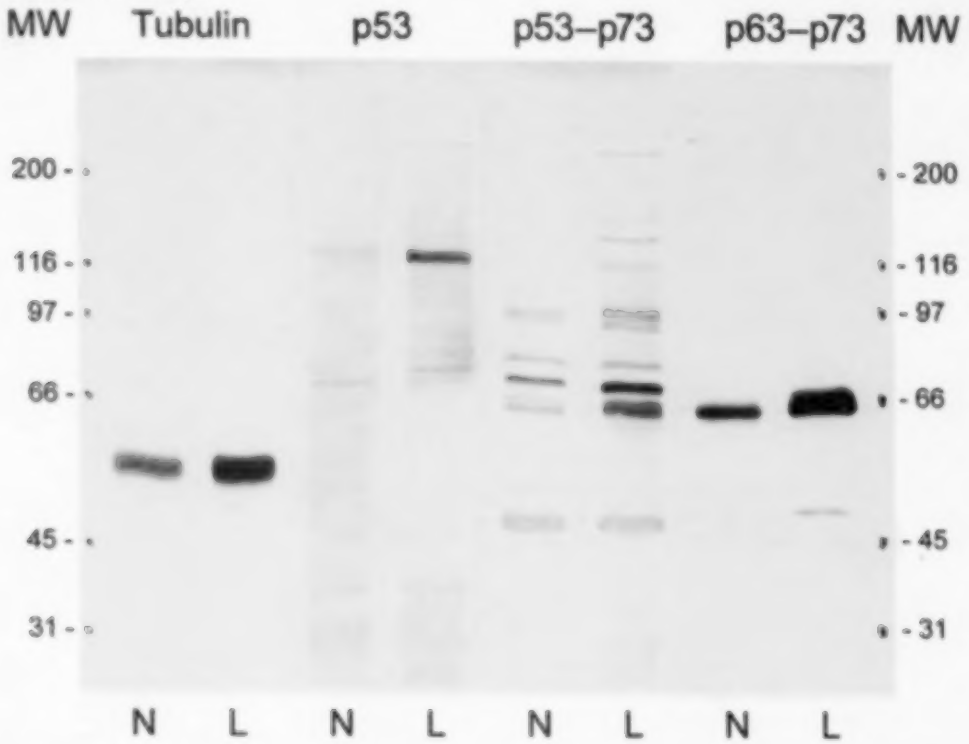
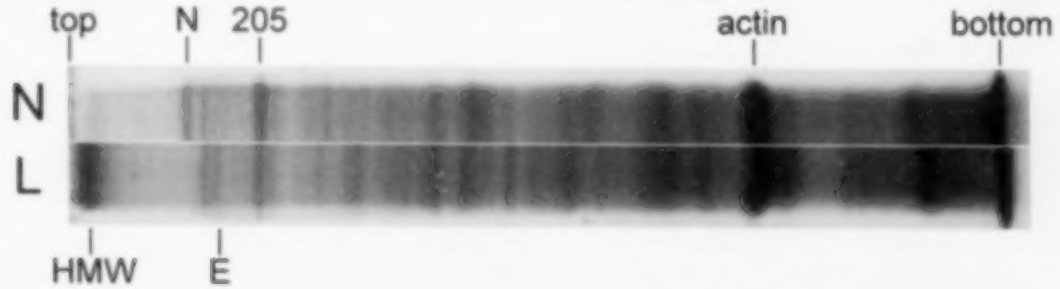


Fig. 4. Direct comparison of normal and haemocytic leukemia cell proteins at equal protein load by low-cross linked SDS–PAGE (8%T/1%C). Lane N, normal haemocytes; lane L, haemocytic leukemia cells; HMW, very high molecular weight protein(s); N and E, proteins characteristic of normal haemocytes and haemocytic leukemia cells, respectively.



bulk of the raw sewage plume. Following a 6-month deployment, the mussels were recovered and brought to the field station. Any moribund or dead mussels were discarded. Haemolymph was subsequently analyzed as described in the Materials and methods. Mussels caged outside Pictou Harbour showed no haemocytic leukemia (MER) or a low prevalence of haemocytic leukemia (LP, 10%). Based on previous work (Reinisch et al. 1984), 10% is considered background. Mussels caged for 6 months in the influence of raw sewage (PS1, PS2, PS3, and PS5) and bleached kraft pulpmill effluents (A) showed significantly elevated haemocytic leukemia levels compared with mussels caged at Merigomish (KW = 106.176, $p < 0.001$ with 13 df; $0.05 < p < 0.10$ multiple range test) (Table 2). At each of those sites, the proportion of mussels with haemocytic leukemia was at least 23% with a maximum of 56% at PS3, similar to the levels observed in the nearby wild population (34%) (results not shown) (Table 2). Each haemolymph sample recorded as positive contained at least 50% cells that were specifically reactive with monoclonal antibody 1E10. These data show

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Table 1. Comparison of the reactivity of normal and leukemia *Mytilus edulis* and *Mya arenaria* hemocytes on immunoblots (*Mya* based on Stephens et al. (2001) and Kelley et al. (2001); *Mytilus* based on this study).

	Normal	Leukemia
Mya arenaria		
1E10	–	+
2A4	+	–
p53	+	+
p63	–	+
p73	–	+
p97	+	–
Mytilus edulis		
1E10	–	–
2A4	–	–
p53	nd	
p63	±	+
p73	±	+
p97	±	+
p120	±	+

Note: +, positive test; –, negative test; nd, not detectable; ±, low.

that mussels placed in sites with industrial contaminants or municipal wastes had a statistically higher chance of developing haemocytic leukemia than mussels caged in clean sites.

Discussion

Bivalve molluscs develop proliferative anaplastic cells in the haemolymph that, in advanced stages, kill the host. As indicated in the Introduction, there is no clear agreement among experts concerning nomenclature, pathogenesis, ontogeny, or even the etiology of this unique disease. We have modified our use of leukemia to include the word haemocytic as recently suggested in correspondence from the Registry of Tumors in Lower Animals. Resolution of the exact diagnosis of this disease will require much more research using monoclonal antibodies that recognize distinct stages of differentiation among haemocyte subsets. Most importantly, the ontogeny of haemocytic leukemia cells will be elucidated by describing epitopes shared by both normal and fully differentiated tumour cells. The definition of individual genes and gene families critically important in the development and differentiation of subsets of cells will also resolve many questions concerning the ontogeny of end-stage tumour cells.

Haemocytic leukemia in *M. edulis* has been thoroughly documented throughout Canada, the United States, and Europe. The haemocytic leukemia cells of *Mytilus* share many phenotypic characteristics with haemocytic leukemia cells of *Mya*. Initially, a murine monoclonal antibody of *Mytilus* was developed by Noel et al. (1991). However, we used a different monoclonal antibody (1E10) to detect and characterize *Mytilus* haemocytic leukemia because the original monoclonal reagent is no longer available. Although 1E10 was initially generated to haemocytic leukemia cells of *M. arenaria*, confocal microscopy with extensive controls showed that 1E10 also specifically reacts with end-stage *Mytilus* haemocytic leukemia cells. In contrast, there is no reactivity with normal *M. edulis* haemocytes. In fact, preliminary data from the laboratory show that 1E10 also reacts specifically with haemocytic leukemia cells but not normal haemocytes from of *M. trossulus* from Vancouver, British Columbia.

Comparative protein analyses show that haemocytic leukemia cells from *Mytilus* characteristically contain a 250-kDa protein that is the likely equivalent of the *Mya* 1E10 antigen. The function of this 250-kDa protein relative to the 300-kDa protein (N) characteristic of normal *Mytilus* or *Mya* haemocytes is unknown, although these may be reciprocally involved in cell adhesion. We will develop monoclonal antibodies to these distinctive proteins as well as to *Mytilus* haemocytic leukemia cells to further refine our ability to detect *Mytilus* haemocytic leukemia in the field.

On average, a given volume of haemolymph from *Mytilus* leukemic individuals yielded about 25 times as much cell protein as that from normal individuals. The corresponding normalized protein profiles closely resembled those already reported for 1E10–2A4 protein identification in *M. arenaria* (Stephens et al. 2001) haemocytes: the high molecular weight protein (N) found only in normal cells, the slightly lower molecular weight protein (E) found predominantly in haemocytic leukemia cells, plus the differential presence of a variety of additional smaller proteins of correlated size. However, the putative *Mytilus* equivalents of Mya 1E10 and 2A4 antigens (proteins E and A) were not cross-reactive after blotting, suggesting species differences or possible denaturation and neither were significantly glycosylated, which may modify the epitope during renaturation. It is not uncommon for antibodies to cross-react with native proteins in cells but not with denatured proteins on blots or vice versa (e.g., the p53 family antibodies reported here).

Conversely, the lack of glycosylation and the fact that a 1E10 cross-reactive antigen in *Mytilus* can be detected in vesicles by immunofluorescence suggests that these cells are in the process of secreting the 1E10 surface antigen seen in fully expressed *Mya* haemocytic leukemia. Also as in *Mya*, the implication of increased mitotic activity is corroborated by the markedly greater tubulin content of haemocytic leukemia cells, but again, not to the same extent as in *Mya*, suggesting cells not yet at end stage. Comparative protein analysis, while valuable in initially documenting characteristic proteins at two extremes, is not particularly useful in evaluating cell populations in transition where the nature and number of individual cells must be determined. Clearly, more research is needed to establish which approach among immunofluorescence, immunoblots, and SDS–PAGE will ultimately be better suited for enviromental health assessments. Perhaps with the development of specific *Mytilus* MABs, and hence their potential greater reproducibility, immunofluorescence will be more suitable. However, strong arguments can also be put forth in favour of the later two approaches.

Western blot analyses demonstrate a significant similarity of *Mytilus* to *Mya* in that leukemic cells differentially express p63–p73 family members. This finding is particularly obvious with p63, a protein that has been associated with haemocytic leukemia (Yamaguchi et al. 2001) and stem cell proliferation (McKeon 2004). p73 has also been associated with malignant haematopoiesis (Freeborn et al. 2003). We

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Fig. 5. (A) Normal haemocyte, leukemic haemocyte, and a 1:1 mixture loaded at equal actin concentrations and analyzed by low-cross linked SDS–PAGE. Characteristic high molecular weight proteins distinguish normal (N) from haemocytic leukemia (E and E′) haemocytes. A major 205-kDa protein of unknown identity is common to both. Proteins E and E′ are the size equivalents of antigen 1E10 in *Mya arenaria*; A denotes a protein that is predominant in normal cells, the near size equivalent of antigen 2A4 in *Mya*. Asterisks denote other lower molecular weight proteins that distinguish the two cells types, also found in *Mya* normal versus haemocytic leukemia cells. (B) Magnification of the upper portion of Fig. 5A. (C) Densitometric traces of the lanes in Fig. 5B normalized to the position and amount of the common 205-kDa protein Solid line, haemocytic leukemia cells; dotted line, normal cells; dashed line, 1:1 mixture.

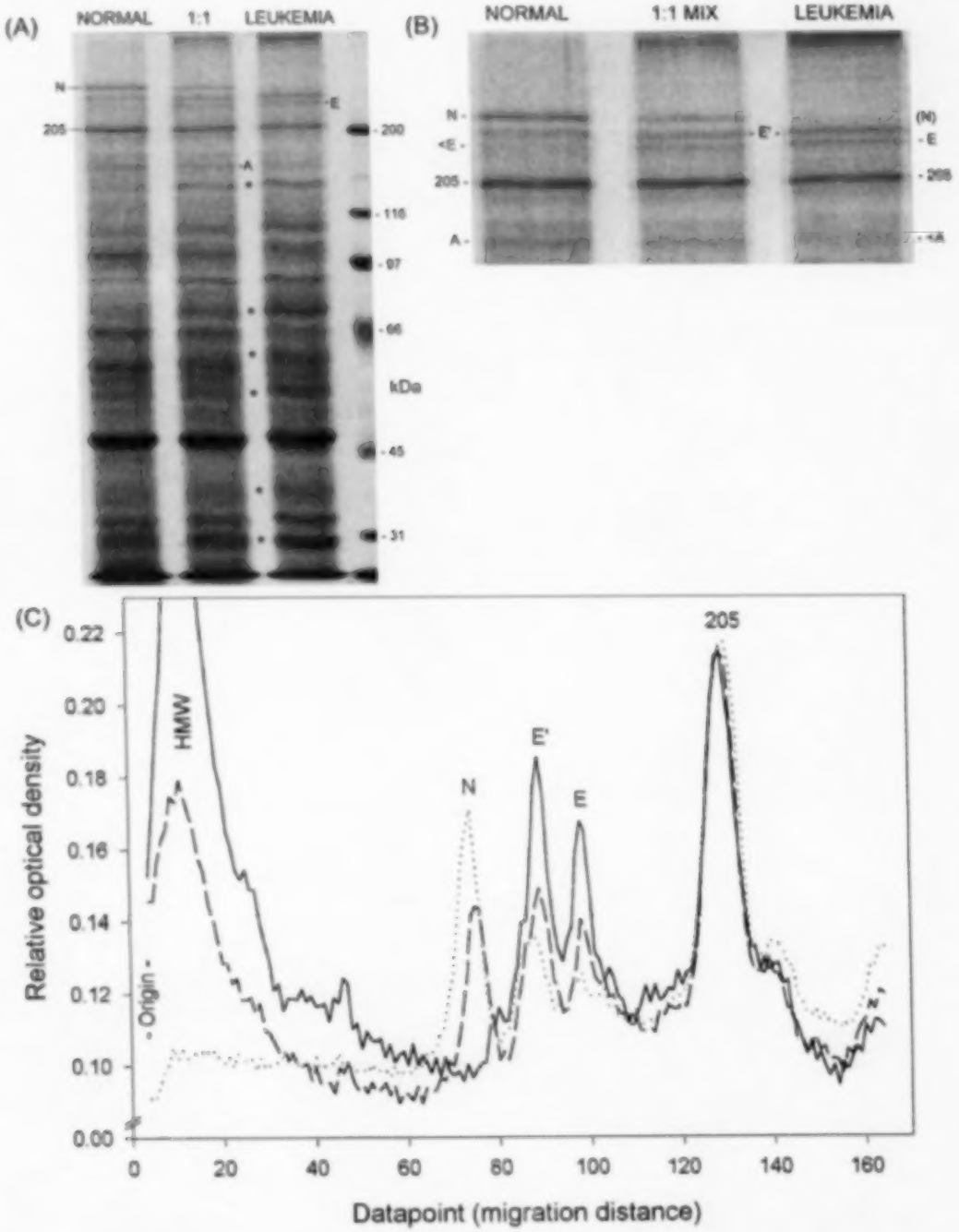

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Table 2. Prevalence of leukemia in *Mytilus edulis* as obtained through visual readings with associated statistical results from a nonparametric two-tailed multiple comparison test using ranks and comparing all sites with Merigomish (MER) independently.

Site	*N*	No. of animals leukemic	Prevalence	Rank	SE	*Q*	$Q_{0.05,13} = 2.666$	$Q_{0.1,13} = 2.424$
PS1	28	7	25	7 913.5	26.7454	2.435		×
PS2	50	13	26	14 320.5	26.7228	2.578		×
PS3	52	29	56	1 947.2	26.7154	5.875	×	
PS4	62	5	8	14 846.5	26.7218	0.822		
PS5	33	10	30	9 900.5	26.735	3.086	×	
MP	29	4	14	6 467.5	26.7567	0.206		
STMW	30	3	10	7 442	26.7486	1.143		
NG	42	8	19	11 531.5	26.7296	2.135		
LH	30	3	10	7 306.5	26.7496	0.974		
Pulpmill A	30	9	30	8 869.5	26.7399	2.923	×	
Pulpmill B	30	7	23	8 407.5	26.7425	2.346		
PP	30	4	13	7 761.5	26.7464	1.541		
LP	30	3	10	7 306.5	26.7496	0.974		
Ref (MER)	30	0	0	6 525	26.7562			

Note: × represents significantly elevated from reference.

expect that we will continue to detect p63–p73 as we explore more haemocytic leukemias within molluscan species.

In terms of molecular markers, the p53 gene family is ideal for investigating specific genetic targets of industrial contaminants in the field. This possibility was initially raised by Bhaskaran et al. (1999) who proposed using p53 as a genotoxic target in fish. Relative to molluscs, our work in New Bedford demonstrated that mutations in the p53 gene were detected when haemocytic leukemia cells of *Mya* were retrieved from a site heavily contaminated with polychlorinated biphenyls (Barker et al. 1997).

Why, in contrast with *Mya*, the different antibodies used do not detect a 53 kDa p53 in immunoblots of *Mytilus* normal or haemocytic leukemia cells is unclear. The most obvious explanation is that the various antibodies used were not sensitive enough to detect low levels of p53 protein, although they will detect p53 in *Mytilus* tissues (data not shown). Secondly, while p53 mRNA may be abundant (Muttray et al. 2005), synthesis may be diminished or the protein degraded. Alternatively, p53 may be posttranslationally modified by ubiquitination (Brooks and Gu 2004) or SUMOylation (Minty et al. 2000; Cox et al. 2003), which is consistent with our detection of higher molecular weight forms.

Precisely what the leukemogenic agents are at the various sites in Pictou Harbour remains to be determined. The rapid induction of leukemia in caged bivalves in situ possibly resulting from the exposure to industrial effluents is an important finding and the development of disease in the caged individuals could reflect the overall health of the immediate aquatic environment. Although there is a possibility that a viral agent could have induced the haemocytic leukemia, the proximity of the cage to the different point sources coupled with the different levels of haemocytic leukemia found in cages closely situated (e.g., MP and PS2) strongly suggests that industrial and (or) municipal waste was the most likely source for the haemocytic leukemia induction. Marine invertebrates such as *M. edulis* are excellent monitors of the environment, since they are sessile filter feeders. Moreover, the fact that these animals develop leukemia within 6 months makes them ideal candidates to study molecular and cellular mechanisms of environmental carcinogenesis within a relatively short period. In our current work, we have deployed more mussels at fewer sites to enable in-depth diagnostics using 1E10, and newer MABs are being developed. Clearly, industrial wastes induce major changes in *Mytilus* including increased microbial infections (Tay et al. 2003) and concomitant suppressed haemocyte function (St-Jean et al. 2003).

Relative to molecular analyses, both the *Mytilus* and *Mya* models strongly suggest that the upregulation of p63–p73 is far more important in the leukemic process than is p53 expression. As our research expands into Vancouver Harbour using *Mytilus*, we will continue to focus on the expression of the entire p53 gene family with the expectation that p63–p73 will be diagnostically upregulated in end-stage haemocytic leukemia cells. As our data reveal, molluscan tumours continue to provide extremely valuable clues not only about environmental health but also about molecular mechanisms controlling cell transformation.

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Genetic influence of parr versus anadromous sires on the life histories of Atlantic salmon (*Salmo salar*)

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Abstract: The potential for sire size to influence life history patterns from fry to sea age at maturity was established by rearing individually identified half-sibs of a single dam and either small (parr) or large (anadromous) sires in a common tank environment. Male sexual maturity was higher among progeny of small versus large sires at both parr (age 0+, 50% versus 32%, $p = 0.065$) and grilse stages (age 2+, 23% versus 10%, $p = 0.034$). In contrast, rates of female grilse (6.8%) and smolt age 1+ (>95%) were independent of sire size. Logistic regression indicated that grilse of small sires had an asymptote body weight (BW) 30% lower than grilse of large sires (1.0 versus 1.4 kg). In comparison, repeated measures analysis of BW, fork length (FL), and condition factor (CF) indicated that growth changes associated with grilsing were independent of sire size and sex. The highest-order interaction for which sire size was significant was day × sire × sex ($p < 0.034$). Independent of sire size, sexual maturation at both age 0+ and age 2+ was associated with a high CF, whereas smolting at age 1+ was associated with a high BW and FL but low CF.

Résumé : Nous avons vérifié la possibilité que la taille du père influence les patterns du cycle biologique depuis le stade alevin jusqu'au stade marin à maturité en élevant individuellement des demi-frères et des demi-soeurs d'une même mère, mais d'un père de petite (tacon) ou de grande (anadrome) taille, dans un environnement d'aquarium commun. La maturité sexuelle des mâles est plus fréquente chez la progéniture des pères de petite taille que chez celle des pères de grande taille, tant au stade tacon (âge 0+, 50 vs 32 %, $p = 0{,}065$) qu'au stade madeleinau (âge 2+, 23 vs 10 %, $p = 0{,}034$). En revanche, les pourcentages de madeleinaux femelles (6,8 %) et de saumoneaux d'âge 1+ (>95 %) sont indépendants de la taille du père. Une régression logistique indique que les madeleinaux nés de pères de petite taille ont une masse corporelle (BW) à l'asymptote de 30 % inférieure à celle des madeleinaux issus de pères de grande taille (1,0 vs 1,4 kg). Par comparaison, des analyses répétées des mesures de BW, de la longueur à la fourche (FL) et du coefficient de condition (CF) indiquent que les changements de croissance associés à la transformation en madeleinaux sont indépendants de la taille du père et du sexe. L'interaction d'ordre le plus élevé dans laquelle la taille du père est significative est celle du jour × père × sexe ($p < 0{,}034$). Quelle que soit la taille du père, la maturation sexuelle aux âges 0+ et 2+ est associée à un CF élevé et la transformation en saumoneau à l'âge 1+ est reliée à des valeurs élevées de BW et de FL, mais à des valeurs faibles de CF.

[Traduit par la Rédaction]

Introduction

A feature of the variable life history patterns exhibited by Atlantic salmon (*Salmo salar*) is the co-existence of two phenotypic forms of sexually mature males, small (<15 cm fork length, FL) and large (>35 cm FL). Small males are freshwater resident parr as young as 1+ years old in the wild and age 0+ in culture (Bailey et al. 1980). Large males, and typically all females, are anadromous, migrating to sea following smolting, returning after 1 year (grilse), or more (multi-sea winter, MSW) to their natal river (Fleming and Reynolds 2004). At spawning, the eggs from each female will typically be fertilized by both small and large sires, creating half-sib families (e.g., Taggart et al. 2001). How sire size affects the life-history patterns of Atlantic salmon is unclear and little studied, overshadowed by the strong influence of environment on somatic growth and the threshold traits of smolting and sexual maturation (Hutchings and Jones 1998; Thorpe et al. 1998; Fleming and Reynolds 2004).

Good evidence of the heritability of male parr sexual maturity comes from two breeding studies in which progeny of large and small sires were reared in a common environment (Thorpe et al. 1983; Gjerde 1984). Faster growth during the alevin stage among half-sib progeny of small versus large

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sires led Garant et al. (2002) to speculate a higher incidence of mature parr age 0+ among the former, based on the rule that fast growth is associated with early maturation (Hutchings and Myers 1994; Thorpe et al. 1998). In contrast, Glebe et al. (1980) concluded that there was no genetic difference between large and small sires judging from the similar incidence of mature parr among their progeny. In the post-smolt marine phase, the consensus is that sire size has no genetic influence on the anadromous age and size at maturity (Nævdal 1983; Gjerde 1984; Glebe and Saunders 1986), although earlier work suggested a link (Schiefer 1971; Schaffer and Elson 1975).

The strong environmental component controlling Atlantic salmon life history patterns is irrefutable. Models describe how individuals with a body size, or state, exceeding a specific genetic threshold for either sexual maturation in spring or smolting in autumn, go on to complete those traits about 6 months later. The threshold for maturation is associated with fatness, and that of smolting with a minimum FL, with some controversy as to whether smolting is a positive or a negative decision (Hutchings and Myers 1994; Thorpe et al. 1998). The potential heritable influence of sire size on these alternate life history patterns was downplayed when game theory indicated alternate phenotypes could co-exist within a population having the same underlying genetic make-up (Gross 1996; Fleming and Reynolds 2004). But clearly, a heritable basis to alternate life history patterns must exist (Hazel et al. 1990; Roff 1996; Gross and Repka 1998), and needs to be defined. For Pacific salmon (*Oncorhynchus* spp.), Heath et al. (2002) has made good progress. Tagging individuals at the fry stage allowed us to test the hypothesis that sire size alters the set point of genetic thresholds of life history traits, detectable as differences in somatic growth around a decision period. Using the same approach, family differences in maturity rates in autumn were linked to body condition the previous winter (Herbinger and Newkirk 1990). Environmental and non-genetic maternal influences were eliminated by comparing half-sibs reared together.

Materials and methods

In August 2000, about 80 returning adults to the Margaree River (Cape Breton, Nova Scotia) were captured randomly and transferred to Margaree Fish Hatchery. On 7 November, gametes from a wild MSW female (70 cm FL) and three wild MSW males (>63 cm FL), all selected randomly, were stripped into four containers and transported to Nova Scotia Agricultural College (Truro, Nova Scotia). The eggs were divided into six equal lots, each about 500 eggs. Three lots were fertilized with milt from the three large sires in single-pair matings. The other three lots were fertilized with milt from six small sires that were hatchery-reared age-1+ parr (13.9–15.9 cm FL, Margaree River stock), allowing milt from two parr per egg lot. The small sires were half-sibs, progeny from a single wild MSW female (92 cm FL) and milt from two wild male grilse mixed together (49.0–56.5 cm FL). Fertilization rate was >99%. At eying, eggs from the two sire sizes were moved into two separate incubators under identical rearing conditions. At hatch (February 2001), the alevins from the three egg lots in each incubator mixed together. The temperature was 5.5 °C to mid-March, then raised to 8 °C, then at swim-up in mid-April raised 1 °C per day to 15 °C. The photoperiod was a simulated seasonal cycle (Latitude 45°N). Fish were fed an excess of commercial salmon diet (Ewos Vextra). In late June 2001, when mean body weight (BW) was approximately 5 g, 200 fish from each sire size group were individually identified with a passive integrated transponder (PIT) tag (Biomark TX1400L, Meridian, Idaho). All fish were then reared together in a single tank (1500 L) with a flow-through supply of freshwater. As a back up, an extra 200 fish were PIT-tagged and reared in a second tank under identical conditions. Water temperature was constant at 15 °C to October and then gradually reduced and held at 6.5 °C from November to May, the lowest possible at our site. On 14 February 2002, 15 fish of each sire group were added to the experiment from the back-up tank; all were males that had matured at age 0+ and were still running milt. The rationale for this was to get a better assessment of maturity at age 0+ on future development. On 15 May, all fish were transferred from freshwater to an adjacent seawater recirculation system at 30 ppt salinity and 8 °C. The system had temperature control and eight rearing tanks (each 1 m in diameter, 500 L) sharing the same water supply (for details see Duston et al. 2004). Only eight mortalities occurred over the initial month in seawater: six parr (mean FL 11.6 cm, BW 17.5 g) and two smolts (15.7 cm FL, 37.0 g BW). Fish were reared in seawater for 16 months at a stable average temperature of 9.6 °C. Fish remained healthy throughout, although occasional losses occurred as a result of mortality post-handling, escapees, and loss of PIT tags. In September 2003, all fish were euthanized and dissected to determine sex and sexual maturity by examination of the gonads.

FL and BW of each fish were recorded 17 times over 820 days: 27 June 2001 (day 1), 13 August 2001 (48), 11 October 2001 (107), 27 November 2001 (154), 4 February 2002 (223), 21 March 2002 (268), 6 May 2002 (314), 4 July 2002 (373), 19 August 2002 (419), 11 October 2002 (472), 18 December 2002 (540), 12 February 2003 (596), 28 March 2003 (640), 6 May 2003 (679), 25 June 2003 (729), 20 August 2003 (785), and 24 September 2003 (820). The 30 fish added in February 2002 were measured 12 times. Condition factor (CF) was calculated as $CF = 100(BW \cdot FL^{-3})$. The fish were classified according to their incidence of maturity at age 0+, smolting at age 1+, maturity at age 1+, maturity at age 2+, and sex. Fish were judged sexually mature when gentle hand pressure on their abdomen caused gametes to be expressed. Milt could be expressed from mature males from October to February. Outside these dates, all males were immature. Smolts were easily distinguished from parr in May by their relatively large FL, low CF, and silvery flanks.

Statistical methods

Only those fish surviving the entire study were included in the data analysis. Incidence of maturity and smolting was analyzed using the CATMOD procedure with the generalized logits response function (SAS Institute Inc. 1999). When the treatment effect was significant, the proportions were further compared using the contrast statement of the CATMOD procedure. Repeated measures analysis was com-

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Table 1. Incidence of sexual maturity at age 0+ and 2+ and incidence of smolting age 1+ among Atlantic salmon (*Salmo salar*) half-sib progeny of either small or large sires.

		Male		Female	
Trait		Small sire	Large sire	Small sire	Large sire
Maturity age 0+	*n*	50	47	72	73
	%	50.0a	31.9b	0.0	0.0
Smolting age 1+	*n*	65	61	72	73
	%	95.4a	95.1a	100.0a	98.6a
Grilse age 2+	*n*	65	63	72	73
	%	23.1a	9.5b	6.9b	6.8b

Note: Shown is the percentage of the total number *n* that exhibited the trait listed. Within a row, percentages sharing the same letter are not significantly different ($p > 0.1$). The increase in the number of males was due to the addition of mature age-1+ parr in February.

pleted on BW, FL, and CF responses as a four-factor factorial. The four factors were sire size (small or large), sex (male or female, determined at end of study), maturity at age 2+ (Mat2+; mature or immature, determined at end of study), and day. For the analysis of male maturity at age 0+ (Mat0+; days 1–154), since only one level of sex (male) was included, the model used for repeated measures analysis was a three-factor factorial. In repeatedly measured data, since the independence assumption on the error terms for the analysis of variance (ANOVA) of a factorial model was likely to be violated, the error component for each model was assumed to be normally distributed with constant variance, and the dependence expressed with a covariance structure of Σ (Littell et al. 1996, 1998). Based on the Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (SBC; Littell et al. 1996), the most appropriate covariance structure was determined to be compound symmetry (CS) using the mixed procedure in SAS (SAS Institute Inc. 1999). Transforming BW and FL to the power 0.25 was needed to satisfy the assumptions for ANOVA. CF data did not require transformation. The least squares means of the treatment combinations from the highest order significant interaction effects were computed and their letter groupings generated at the 5% level of significance.

Nonlinear regression model fitting

For each of the eight sire by sex by maturity at age-2+ combinations, the least squares means of BW at each of the 17 time points were used to fit a logistic growth model. To compare these eight treatment combinations in terms of the model parameters, an extra sum of squares analysis of a nested nonlinear regression with incremental parameters model was completed. This was done in line with fitting nonlinear and nested nonlinear regression models (Bates and Watts 1988). The analysis was completed using the NLIN procedure of SAS (SAS Inst. 1999), and S-PLUS 6.2 (Insightful Corp. 2003). The functional form of the logistic model is

$$(1) \qquad Y = \frac{\theta_1}{1 + \exp\left(-\frac{x - \theta_2}{\theta_3}\right)}$$

where Y is body weight and x is time (days). The model parameters represent the asymptote (θ_1), the time to half the asymptote (θ_2), and the time interval from θ_2 to about three-quarters of the asymptote (θ_3).

Results

Incidence of sexual maturity and smolting

The incidence of mature male parr in November at age 0+ among the progeny of small sires was 50% (25/50), compared with 31.9% among progeny of large sires ($p = 0.065$; Table 1). Females were immature age 0+. By the following May, the incidence of smolts at age 1+ was over 95% and independent of sire size (Table 1). Only nine fish remained parr, eight of which were males that matured at age 0+ (five large sire, three small sire) and one female (large sire). In autumn at age 1+, sexual maturation in seawater occurred only among six male parr, three from each sire treatment group. All six had also matured at age 0+. By the following autumn at age 2+, the probability that an individual matured as a grilse was dependent on both the size of its male parent and its sex. The male grilse rate among the progeny of small sires was over double that of the large sire group: 23.1 (15/65) versus 9.5% (6/63; $p = 0.034$). In contrast, among females, the incidence of grilse was independent of sire size (Table 1). Among individual males, maturation as both parr (age 0+) and grilse, "re-maturation" was 24% (9/37) among the progeny of small sires compared with 7% (2/27) among progeny of large sires ($p = 0.051$). Males that matured in autumn at age 0+ by the following spring were immature by the completion of smolting in May and were indistinguishable from the rest of the fish. The increase in fish number from 61 to 63 of male progeny of large sires (Table 1) was due to two parr in spring age 1+ that subsequently completed smolting in seawater and grew rapidly.

Somatic growth and male sexual maturation at age 0+ (days 1–154)

During the first 154 days (June–November), sire size had no influence on the growth of either maturing or immature males ($p > 0.471$ for day × sire × Mat0+ for BW, FL, and CF). However, independent of sire size, large differences in growth occurred between immature and maturing males (day × Mat0+ interaction; $p = 0.001$ for BW, FL, and CF). From mid-August onwards, immature males were larger than maturing males in both BW and FL, with over a 10-g difference by November (37.2 versus 26.6 g; Table 2). In contrast,

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Table 2. Body size of sexually maturing age-0+ Atlantic salmon (*Salmo salar*) males (M) compared with immature males (I).

Date (days)	Maturity	BW	FL	CF
27 June (1)	I	5.8g	7.8g	1.19b
	M	4.8g	7.4g	1.19b
13 August (48)	I	15.0e	10.7e	1.19b
	M	12.4f	9.9f	1.26a
11 October (107)	I	30.9b	13.8b	1.17b
	M	20.8d	11.7d	1.29a
27 November (154)	I	37.2a	14.9a	1.12c
	M	26.6c	12.8c	1.26a

Note: Least squares means for body weight (BW; g), fork length (FL; cm), and condition factor (CF) sharing the same letter within columns are not significantly different at the 5% level. For BW, FL, and CF, the two-way interaction (day × Mat0+) was highly significant ($p = 0.001$), but the three-way interaction (day × sire × Mat0+) was not ($p > 0.471$).

the condition factor of immature males was significantly lower than that of maturing males from August onwards (Table 2).

Somatic growth from fry to grilse age 2+ (days 1–820)

The four-way interaction day × sire × sex × Mat2+ was not significant, indicating that the change in body size over the 820-day study of fish that became grilse and those that remained immature at age 2+ was similar, regardless of both sire size and sex. Nevertheless, several main and interaction effects were significant. The highest order interaction for which sire size was significant for all three body size indicators was day × sire × sex ($p \leq 0.034$; Table 3). This three-way interaction effect was significant only because of those males that matured age 0+ (described above). Omitting mature males age 0+ from the analysis resulted in the three-way interaction becoming insignificant ($p = 0.586$, data not shown). The other significant interaction effect for BW, FL, and CF was day × Mat2+ ($p = 0.001$; Table 3).

Day × sire × sex interaction

The initial body size of all four sire–sex combinations was similar (Table 4). By November (day 154), males were smaller on average than females among the progeny of both sire groups as a result of sexual maturation among a proportion of the males, as described above. Immature males and females in November had a similar mean BW, 36.6 and 38.6 g, respectively. Between November and March (days 154–268), mean BW increased about 10 g among all groups as a result of the 6.5 °C temperature and daily feeding. Consequently, >90% of fish completed smolting age 1+, including most of the previously mature males. By early May (day 314), the body size of the smolts was largely independent of sire and sex, except for FL among the progeny of large sires (16.5 versus 18.1 cm; Table 4). CF in May (1.05–1.07) was relatively low, which is typical of smolts (see also Fig. 1).

In seawater, post-smolt BW increased about threefold between May and August (days 314–419), with body size remaining independent of sire and sex (Table 4). However, by December, age-1+ (day 540) females tended to be larger than males, and male progeny of small sires had the lowest mean BW (286 g), FL (28.7 cm), and CF (1.20) (Table 4). By March (day 640), the BW of females was clearly greater than males and independent of sire size. Also, male progeny of small sires had a lower BW and CF than their half-sibs. These trends continued to September age 2+ (day 820), with females remaining largest and the male progeny of small sires (BW, 669 g and FL, 39.4 cm) being smaller than the other three sire × sex combinations (Table 4). Among the male progeny of small sires, those individuals that matured at both age 0+ and 2+ had the smallest mean BW (572 g) (Table 5), but across the 12 sire × sex × maturity groupings, there was considerable similarity in the final BW, as indicated by the shared letter groupings (Table 5).

Table 3. Probability values for all main and interaction effects on the body size of Atlantic salmon (*Salmo salar*) progeny of either large or small sires using the full dataset (days 1–820).

Source of variation	Body weight	Fork length	Condition factor
Day	0.001	0.001	0.001
Sire	0.113	0.326	0.001
Day × sire	0.001	0.001	0.001
Sex	0.001	0.001	0.143
Day × sex	0.001	0.001	0.001
Sire × sex	0.823	0.419	0.101
Day × sire × sex	0.034	0.023	0.023
Mat2+	0.007	0.130	0.001
Day × Mat2+	0.001	0.001	0.001
Sire × Mat2+	0.373	0.289	0.323
Day × sire × Mat2+	0.813	0.292	0.272
Sex × Mat2+	0.602	0.550	0.997
Day × sex × Mat2+	0.999	0.975	0.303
Sire × sex × Mat2+	0.562	0.508	0.917
Day × sire × sex × Mat2+	0.999	0.991	0.830

Day × Mat2+ interaction

Sexual maturation as grilse at age 2+ was associated with significant changes in BW, FL, and CF (all $p = 0.001$; Table 3). Incipient grilse had a significantly higher mean CF than immature fish from early July age 1+ onwards, over a year before completion of maturation (Fig. 1). By comparison, BW and FL of maturing fish were significantly higher than that of immature fish for only a few months during the second season in seawater. By late September, towards the completion of sexual maturation, both BW and FL of grilse and immature fish had converged.

Logistic regression analysis for body weight

Using starting values determined from plots of day versus BW, the logistic model for each of the eight sire × sex × Mat2+ combinations converged quickly. Normal distribution and constant variance assumptions were valid, and from the approximate correlation matrix, the model parameters were not excessively correlated. Together these results indicated that the increase in BW in each of the eight combinations followed the logistic growth model (Fig. 2). Extrapolation of the regression models from the end of the study (day 820) to day 1000 indicated the relatively small predicted size of grilse of both sexes, progeny of small sires (Fig. 2). The estimated asymptote for BW (θ_1) of grilse progeny of small sires was 25%–33% less than that for grilse progeny of large sires

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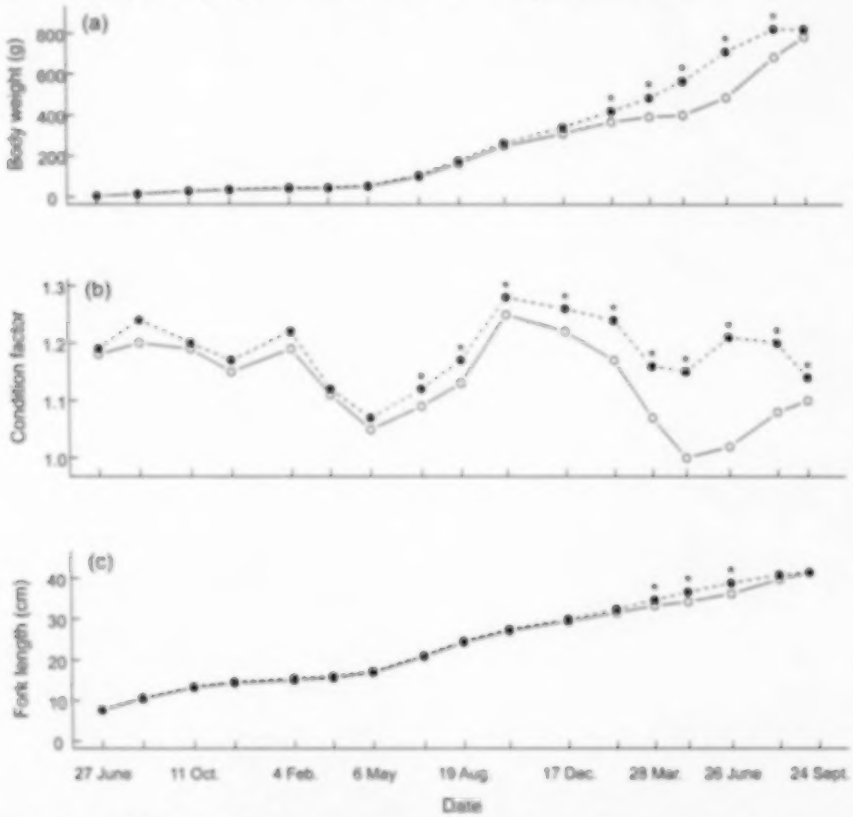
Table 4. The three-way interaction of day × sire × sex on body size of Atlantic salmon (*Salmo salar*) reared in tanks for 820 days, all of which completed smolting at age 1+.

	Body weight				Fork length				Condition factor			
	Large		Small		Large		Small		Large		Small	
Date, age (day)	M	F	M	F	M	F	M	F	M	F	M	F
June, 0+ (1)	4a	6a	5a	5a	7.2a	7.7a	7.7a	7.7a	1.21a	1.18a	1.18a	1.17a
November, 0+ (154)	31b	44a	32b	38a	13.8b	15.6a	14.0b	14.9a	1.20a	1.15ab	1.16ab	1.13b
May, 1+ (314)	48a	63a	48a	54a	16.5b	18.1a	16.6b	17.2ab	1.07a	1.06a	1.06a	1.05a
August, 1+ (419)	178a	167a	167a	161a	25.1a	24.5a	24.2a	24.0a	1.18a	1.13b	1.16ab	1.13b
December, 1+ (540)	312ab	375a	286b	331ab	29.0b	30.8a	28.7b	29.9ab	1.27ab	1.28a	1.20c	1.23bc
March, 2+ (640)	430b	503a	371c	455ab	33.2bc	35.5a	32.5c	34.6ab	1.17a	1.12ab	1.08b	1.09ab
September, 2+ (820)	809a	917a	669b	838a	40.7bc	43.7a	39.4c	42.2ab	1.19a	1.10b	1.09b	1.10b

Note: Shown are the least squares means on selected days for body size of progeny of either large or small sires subdivided by sex. For each variable, values sharing the same letter within rows are not significantly different at the 5% level.

Fig. 1. Comparison of the body size over 820 days of Atlantic salmon (*Salmo salar*) maturing as grilse at age 2+ (- - ● - -) and fish remaining immature at age 2+ (—○—). Shown are least squares means of (*a*) body weight, (*b*) condition factor, and (*c*) fork length pooled by sex and sire size. On a given day, significant differences are shown by an asterisk (*).



for both males (907 versus 1355 g; $p < 0.05$) and females (1095 versus 1445 g; $p < 0.05$; Table 6). Among fish remaining immature at age 2+, their asymptote BW was over 2 kg and independent of both sire and sex (Table 6). The relatively high incidence of male grilse among progeny of small versus large sires was associated with a significantly shorter number of days to reach half the asymptote BW (θ_2), 646 versus 724 days (Table 6). In contrast, among females that matured at age 2+, both the incidence of grilse and the θ_2 values were independent of sire size. Beyond the θ_2 inflection point, maturing fish took 124–151 days to reach three-quarters of the asymptote (θ_3), significantly shorter than immature fish, irrespective of sire size and sex (Table 6).

Discussion

The relatively high incidence of grilse among male progeny of small sires in this lab-based study established that

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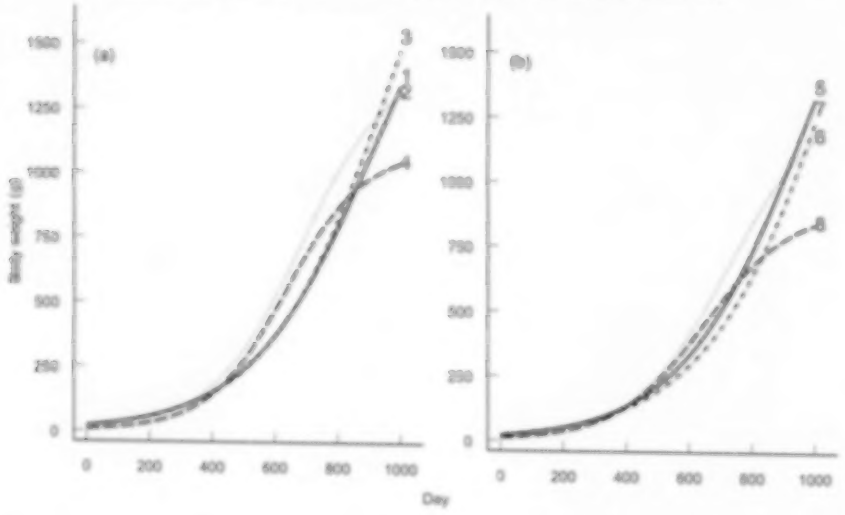
Table 5. Final least squares mean body weight (g) of the progeny of large and small sires subdivided by sex, maturity at age 0+ (Mat0+), and maturity at age 2+ (Mat2+) as grilse.

	Large sire		Large sire				Small sire		Small sire			
	Female		Male				Female		Male			
Mat0+	I	I	I	I	M	M	I	I	I	I	M	M
Mat2+	I	M	I	M	I	M	I	M	I	M	I	M
Final mean BW	845ab	993a	841ab	909ab	699bcd	735abcd	853ab	822abc	726bcd	824abc	654cd	572d

Note: I, immature; M, mature. Means followed by the same letter are not significantly different at the 5% level.

Fig. 2. Fitted logistic growth models for the body weights (g) of sire size by maturity at age 2+ combinations of (*a*) female and (*b*) male Atlantic salmon (*Salmo salar*). Solid lines 1 and 5 are immature, large sire; dotted lines 2 and 6 are mature, large sire; dashed–dotted lines 3 and 7 are immature, small sire; and dashed lines 4 and 8 are mature, small sire.



sire phenotype can have a genetic influence on post-smolt development of Margaree River stock. Among other stocks of Atlantic salmon, both wild and cultured, this genetic influence has remained largely undetected, supporting the hypothesis that male parr sires have no influence on sea age at maturity. The disparity between the present and previous studies is perhaps not surprising, given the strong environmental influence affecting age and size at maturity of adult Atlantic salmon, genetic difference between stocks, and potential interactions between genotype and environment. During the freshwater stage, the results confirmed previous findings that sexual maturation among male parr is heritable. However, differences in growth rates between maturing parr and potential smolts led us to question the dogma that somatic growth and age at maturity are inversely related.

That a connection exists between river and sea age at maturity, as evidenced by the relatively high incidence of mature parr and grilse among progeny of small sires, is in accord with Schiefer (1971) in his study of north shore St. Lawrence River stocks. However, a follow-up study by Bielak and Power (1986) reaffirmed the hypothesis of independence between river and sea age at maturity. Similarly, other studies found male parr sires had no genetic influence on the sea age at maturity of pen-reared Atlantic salmon (Nævdal 1983; Gjerde 1984; Glebe and Saunders 1986). In cage conditions, winter seawater temperatures as low as 1 °C have been associated with a lower incidence of grilse, emphasizing the strong environmental influence on sea age at maturity (Saunders et al. 1983). In contrast, we held seawater at 9–10 °C for the entire post-smolt period, which might have improved our chances of detecting a sire effect. Despite this attempt to promote growth and sexual maturation, both overall grilse rate and body size were about half that reported for wild Margaree fish (Claytor and Chadwick 1985; Chaput and Claytor 1988). We believe the small tanks (1 m in diameter) inhibited growth of the fish beyond 25 cm FL or so. However, a new finding was the inhibitory influence of small sires on post-smolt growth, as evidenced by both the significant three-way interaction (day × sire × sex) detected by repeated measures analysis and logistic modeling that predicted small grilse. This effect was peculiar since it occurred after August age 1+, a time when post-smolt body size of all sire–sex combinations were more or less equal, following compensatory growth among males following transfer to seawater in May. Skilbrei (1990) also reported compensatory sea growth among males that previously matured as parr. In the wild, if parr sires contributed significantly to the incidence of grilse, the logistic regression modeling predicts two size classes among the male grilse. From the Margaree River, 40 years of data on the reported size of angled grilse appears skewed from normal, but bimodality is not evident (Chaput and Claytor 1988).

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Table 6. Logistic growth model parameters for the eight treatment combinations of Atlantic salmon (*Salmo salar*).

Treatment combination	Sex	Sire	Mat2+	θ_1	θ_2	θ_3
1	F	Large	Immature	2250a	923a	200a
2	F	Large	Mature	1445b	682bc	143b
3	F	Small	Immature	2807a	979a	200a
4	F	Small	Mature	1095c	636bc	124b
5	M	Large	Immature	2523a	980a	202a
6	M	Large	Mature	1355b	724b	151b
7	M	Small	Immature	3480a	1133a	224a
8	M	Small	Mature	907d	646c	140b

Note: θ_1 is the asymptote for body weight. θ_2 and θ_3 are the number of days to reach half and half to three-quarters of the asymptote, respectively. Parameter estimates followed by the same letter are not significantly different at the 5% level.

The high ratio of male to female grilse we observed is typical of the species (Gjerde 1984). Grilse returning to the Margaree River are 94% male (G. Chaput, Department of Fisheries and Oceans, P.O. Box 5030, Moncton, NB E1C 9B6, unpublished data).

The hypothesis that higher grilse rates among the progeny of small sires was due to a lower threshold body size for the decision to mature received support from the logistic modeling of body weight. The decision period for becoming a grilse or remaining immature is likely made during the first winter in seawater (Thorpe et al. 1998; Duston and Saunders 1999), perhaps November–February (days 500–600) of this study. The divergence in BW at about day 500 between male incipient grilse of small versus large sires, coupled with the significantly different time to half asymptote θ_2, suggests a lower body size threshold for grilse among parr sired males. Evidence of differences in threshold body sizes for grilsing, specifically CF in winter, was found between families of Atlantic salmon, but sire size was not investigated (Herbinger and Newkirk 1990). In contrast to the logistic modeling, the repeated measures analysis indicated the somatic growth changes associated with grilsing were affected neither by sire size nor sex. Their contrasting outputs illustrate the importance of analyzing data using different statistical methods to gain a better understanding of the complexities of nature. From the two-way day × Mat2+ interaction, CF was the earliest indicator of maturation in July as post-smolts, confirming Duston and Saunders (1999). The temporal changes in CF between maturing and immature fish at constant 10 °C followed a similar pattern to Atlantic salmon at ambient water temperature (Duston and Saunders 1999), with the biggest divergence occurring March–June, about 6 months prior to spawning season. Studies on wild Atlantic salmon have detected an increased growth of incipient grilse during late summer (Friedland and Haas 1996) and winter (Friedland et al. 1998), based on scale reading and strontium–calcium ratios, respectively. In the present study, we suggest that the difference in BW and FL between maturing and immature fish was a consequence rather than cause of sexual maturation as a result of the timing of the divergence (Duston and Saunders 1999).

As for sexual maturation among male parr at age 0+, the results support the hypothesis that sexual maturation among male parr is heritable. In autumn at age 1+ among the progeny of small versus large sires, Gjerde (1984) reported 6.9% versus 0% male maturity, and Thorpe et al. (1983) found 25% versus 15% maturity for the population (males and females), the latter percentages comparable to our data for age-0+ males. In contrast, Glebe et al. (1980) reported the incidence of parr maturity was largely independent of sire size, leading them to conclude that there was no genetic difference between anadromous and parr sires. Whenever such a null hypothesis stands, it can be argued that environment, given its large influence on salmonid life histories, prevented any underlying genetic effects from being detected. Alternatively, Thorpe et al. (1983) suggested that the large sires used by Glebe et al. (1980) had themselves matured as parr and thus would not be qualitatively different from small sires. This is possible as sexual maturation does not preclude smolting but can inhibit it (Saunders et al. 1994; Whalen et al. 2000).

Sire size had no influence on the growth pattern of underyearlings, thus providing no support for the hypothesis that sire size influences parr maturity by altering a body size threshold for maturation. Nevertheless, insight was gained into the relationship among growth and parr maturation and smolting. Male sexual maturation at age 0+ is common among cultured Atlantic salmon (Bailey et al. 1980; Saunders et al. 1994) but rare in nature because of the poorer growing conditions (Letcher and Terrick 1998). Among wild parr, sexual maturity at age 1+ is prevalent, with the incidence of maturity positively related to body size in late winter and growth opportunity early in the growing season (Rowe and Thorpe 1990; Berglund 1995; Whalen and Parrish 1999). Accordingly, a central tenet of models describing salmonid life history variation is that somatic growth and age at maturity are inversely related (Hutchings and Myers 1994; Thorpe et al. 1998). More controversially, Thorpe et al. (1998) argue the evolutionary priority of an individual is to sexually mature and that smolting is a negative developmental decision. This latter idea has its critics (e.g., Hendry et al. 2004) and is not supported by our data. The relative fast growth, in terms of both BW and FL, of immature males compared with maturing males through to November age 0+ after starting the same body size in June indicates smolting at age 1+ is a positive developmental decision. Others have also questioned whether fast growth and early maturation are always connected (Saunders et al. 1982;

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Bohlin et al. 1990), making it unwise to speculate on outcomes based on short-term differences in growth (e.g., Garant et al. 2002). From the perspective of CF, maturation at age 0+ can be reconciled with general models: high CF was associated with sexual maturation (Thorpe et al. 1998). Overall, it appears we encountered two discrete thresholds: one for smolting at age 1+, attainable by the top performers attaining a certain FL at a certain time; and one for sexual maturation at age 0+, achievable by the smaller males with a relatively high CF.

The high incidence of smolts at age 1+, including males that matured at age 0+, can be attributed to the good growth conditions through winter, as found previously (Duston and Saunders 1997). As such, sire size had no effect on the incidence of smolts, supporting previous findings (Jones and King 1950; Thorpe and Morgan 1980). In contrast, for fish exposed to harsh winter conditions in the wild, small sires could reduce smolt production as a consequence of their progeny exhibiting a relatively high incidence of sexual maturity as parr and then suffering high mortality over winter. Toward the northern limit of the species range, the male–female smolt ratio can be as high as 1:5 (Lee and Power 1976; Myers 1984). Further south, overwinter survival of wild mature parr was similar to that of immature parr, about 33%, but their probability of smolting was threefold lower, because of a smaller body size (Whalen et al. 2000). In the Margaree River, the majority of smolts are female (73% of 641 smolts sampled between 2001 and 2004; G. Chaput, Department of Fisheries and Oceans, P.O. Box 5030, Moncton, NB E1C 9B6, unpublished data), which is likely a consequence of a proportion of mature male parr (≥age 1+) failing to smolt.

In conclusion, parr sires can influence somatic growth and both freshwater and sea age at maturity, at least under lab conditions. Whether these effects are of any significance in the wild requires environment to be included in experimental designs to gain insight into the reaction norms that permit the co-existence of anadromous and non-anadromous life-history patterns. At the very least, the results show the potential for sire size to play some role in the wild, and increase our awareness of the possible complexities.

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Impacts of hydraulic dredging on a macrobenthic community of the Adriatic Sea, Italy

E.B. Morello, C. Froglia, R.J.A. Atkinson, and P.G. Moore

Abstract: Hydraulic dredging that targets the bivalve *Chamelea gallina* in the northern and central Adriatic Sea (Italy) has been taking place for over 30 years. Seventy-three commercial dredgers harvest the resource within the sandy coastal area of the Ancona Maritime District (central Adriatic Sea). Despite this chronic disturbance, studies aimed at investigating the impacts of the fishery on the macrobenthic community of the area have never been carried out. To remedy this, sampling was accomplished within an area of the District from which hydraulic dredging was banned, within the framework of a balanced beyond-BACI (before/after, control/impact) experimental design. Data regarding seven groups of species were analysed separately by means of permutational multivariate analysis of variance. No impacts attributable to hydraulic dredging were found upon consideration of the entire sampled macrobenthic community, the Polychaeta, the Crustacea, detritivores, and suspensivores. In contrast, a sustained press impact of fishing was revealed for the Mollusca, and the bivalve *Abra alba* was found to be particularly susceptible. *Abra alba* was suggested as a possible impact indicator. A short-lived pulse impact on the predator and scavenger trophic guild was observed and was limited to the 1st sampling day after experimental hydraulic dredging.

Résumé : Depuis plus de trente ans, il se fait du dragage hydraulique dans le nord et le centre de l'Adriatique (Italie) pour la récolte du bivalve *Chamelea gallina*. Soixante-treize pêcheurs commerciaux font des opérations de dragage pour recueillir cette ressource dans la région côtière sablonneuse du district maritime d'Ancona (région centrale de l'Adriatique). Malgré cette perturbation chronique, il n'y a jamais eu d'études d'évaluation des impacts de ces récoltes sur la communauté du macrobenthos dans la région. Pour remédier à cette carence, nous avons entrepris un échantillonnage dans une région du district maritime où le dragage hydraulique a été interdit dans le cadre d'une étude à plan d'expérience de type « au-delà de BACI (avant/après, témoin/impact) » équilibré. Nous avons analysé séparément les données sur sept groupes d'espèces au moyen d'une analyse multidimensionnelle de variance avec permutations. Nous n'avons trouvé aucun impact dû au dragage hydraulique lors de l'analyse de la faune entière de macrobenthos échantillonnée, ni lors de celles des polychètes, des crustacés, des détritivores et des suspensivores. En revanche, il y a un impact de « pression soutenue » de cette pêche sur les mollusques et particulièrement sur le bivalve *Abra alba* qui s'est révélé très vulnérable. Nous proposons donc l'utilisation d'*A. alba* comme espèce indicatrice des impacts potentiels. Nous avons observé un impact de courte durée de type pulsation sur les guildes trophiques des prédateurs et des éboueurs qui est restreint au premier jour d'échantillonnage suivant un dragage hydraulique expérimental.

[Traduit par la Rédaction]

Introduction

Bottom fishing is the most widespread anthropogenic activity aimed at exploiting the living resources within the marine environment (Jennings and Kaiser 1998). Fishing disturbance is thought to be the main ecological structuring force on the benthos of the North Sea (Hall-Spencer 1999) and has been proposed as the cause of long-term changes within benthic communities (Frid et al. 2000). Bottom-towed gears exploit species that live or feed within the substratum and are therefore designed for maximum contact with the sea bed. They have major ecological effects on the fauna and flora associated with the substratum that lead to changes in habitat and community structure and initiate shifts in the relationships between organisms (Jennings and Kaiser 1998; Frid et al. 2000). Among bottom-fishing activities, shellfish dredging and hydraulic dredging are probably responsible for causing the greatest disturbance of the seabed (Eleftheriou 2000; Kaiser et al. 2002).

Hydraulic dredges scrape the surface of the substratum and dig into it, resuspending significant amounts of sediment. Overall, it has been found that hydraulic dredging contributes to destabilization and partial modification of sediment conditions, which results in an overall decrease in habitat complexity and has direct implications with respect to the benthic community (Brambati and Fontolan 1990;

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Pranovi and Giovanardi 1994; Tuck et al. 2000). Drastic reductions in the abundances of infaunal organisms are widely reported as a consequence of mechanical, suction, and hydraulic dredging (Hall et al. 1990; Pranovi and Giovanardi 1994; Tuck et al. 2000). Shifts in benthic community structure in favour of few dominant opportunistic species have been observed (Pranovi and Giovanardi 1994). This is a condition that Warwick (1986) associates with disturbance.

The infaunal bivalve *Chamelea gallina* is the target of a large fleet of hydraulic dredgers operating in the sandy coastal bottoms (depth of 3–12 m) of the northern and central Adriatic Sea. This is an area subjected to intense hydrodynamism and environmental fluctuations, and the benthic community associated with it will have an inherent resilience to natural physical disturbance (MacDonald et al. 1996; Kaiser 1998). Thus, the essence of impact studies aimed at quantifying the effects of fishing activities is that of unequivocally detecting ecologically significant responses over and above those induced by natural disturbance and (or) natural spatial and temporal variability due to factors such as contagious spatial distribution and recruitment events (Morrisey et al. 1992*a*, 1992*b*).

The commercial fishery for *C. gallina* in the Adriatic Sea has been taking place for over 30 years and the resource is now showing strong signs of depletion and overexploitation, calling for immediate management actions (Froglia 2000). Despite this chronic disturbance, impact studies have never been carried out in the area. This study describes a small-scale experimental approach to evaluating any impacts on the benthic community through a beyond-BACI (before/after, control/impact) experimental design (Underwood 1991, 1992, 1994). Several authors have reported the weakness of univariate analyses carried out on summary statistics, such as species richness and diversity, in detecting changes within a disturbed community or assemblage and have proposed multivariate species-dependent analyses to be more sensitive to changes in community structure parameters such as dominance (Colangelo et al. 1996; Schratzberger et al. 2002). For these reasons, the choice was made to apply non-parametric multivariate analysis of variance techniques capable of investigating interactions, as well as single factors (Anderson 2001), to explore the data generated by the beyond-BACI experimental design.

Thus, the aims and objectives of this study were as follows: (*i*) quantify and evaluate the impacts of hydraulic dredging on the structure of the macrobenthic community present in the study area; (*ii*) identify groups of taxa or trophic guilds particularly sensitive to this fishing activity; and (*iii*) identify species whose negative (or positive) response to the fishing activity could be used as an indicator of fishing disturbance. Despite the relatively limited spatial and temporal extent of the study, the remarkable uniformity of the coastal zone of interest to hydraulic dredging, the unique nature of the gear, and the rigorous experimental design may allow the information gained to be extrapolated for management purposes.

Material and methods

Study area

This study took place within the central sector of the Adriatic Sea. The substratum is dominated by very fine sands (83.36% average; Hauton et al. 2002) and hosts the biocoenosis of fine, well-sorted sands (SFBC) described by Pérès and Picard (1964) for the Mediterranean. Within this biocoenosis, dominance of the target species of the hydraulic dredging fishery, i.e., *C. gallina*, has lead this area to be termed "facies à *C. gallina*" (Pérès and Picard 1964). The study was carried out within a confined area of the Ancona Maritime District (43°36.9′N, 13°27.8′E; 6 m depth; Fig. 1) from which commercial hydraulic dredging was banned from 4 April 2001 to 1 October 2001.

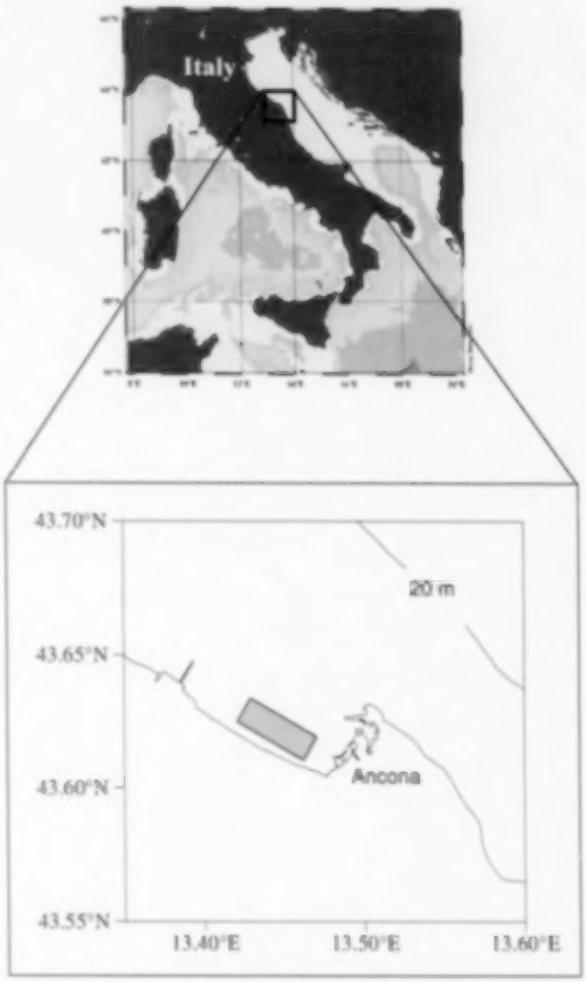

Fig. 1. Location of the study area within the Ancona Maritime District (central Adriatic Sea; latitude and longitude in decimal degrees). Sampled sites were contained within the shaded rectangular box.

Gear

The commercial hydraulic dredge used to harvest *C. gallina* comprises a 2.4–3 m wide rectangular cage weighing 0.6–0.8 tonnes, mounted on two sledge runners to prevent it from digging into the substratum to a depth of more than 4–6 cm. The front of the cage is connected by a hose to a centrifugal water pump that ejects water under pressure (1.2–

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1.8 bar; 1 bar = 100 kPa) from the nozzles at the mouth of the dredge and inside the dredge cage (Froglia and Bolognini 1987). These resuspend sand and organisms immediately ahead of and within the dredge and wash accumulated debris out of the cage. Once suitable fishing grounds are reached (depth of 3–12 m), the dredge is lowered from the bow and the vessel moves astern, warping on a big anchor for a variable distance depending on how much cable is paid out (Froglia and Bolognini 1987), or by using the propeller. At the end of the tow, the cage is hauled onto a metal frame on the bow of the boat and its contents are spilled into a collecting box. The catch is conveyed to a mechanical vibrating sieve that sorts the catch by size classes.

Sampling methods

The impact of hydraulic dredging for *C. gallina* on the macrozoobenthic assemblages inhabiting sandy substrata within the study area was assessed using a balanced beyond-BACI experimental design (Underwood 1991, 1992, 1994). Within this area, four experimental plots or sites (approximately 3 m × 50 m) were chosen at random, hence granting adequate spatial replication (Hurlbert 1984; Underwood 1992). Two such plots were subjected to dredging and the remaining two served as control areas. Dredging treatment was randomly allocated to sites, resulting in dredged and control plots being interspersed within the study area. Each site was visited four times before (14 June 2001, 21 June 2001, 5 July 2001, and 7 July 2001) and four times after (11 July 2001, 14 July 2001, 18 July 2001, and 25 July 2001) the impact (7 July 2001) at random time intervals. The impact was created experimentally with a hydraulic dredge using the anchor method (repeated 50 m tows). Each site was subdivided into 50 numbered subplots. On each visit, three subplots (replicates) per site were chosen randomly and 0.25 m^2 quadrat samples were taken from each using an air-operated suction sampler (collecting bag mesh size = 1 mm), resulting in a total of 96 samples at the end of the experiment. None of the subplots was sampled twice. The samples collected were initially fixed in 5% formalin in seawater and subsequently stored in 75% ethanol. Each sample was sorted under a dissecting microscope. The organisms were identified to species wherever possible, counted, and wet-weighed to milligram accuracy.

Statistical analyses

The resulting experimental design comprised four factors: (1) before/after (B) with two levels (before and after), orthogonal and fixed; (2) time (T) with four levels, nested in B and random; (3) impact (I) with two levels (dredged and control), orthogonal and fixed; and (4) site (S) with two levels, nested in I and random. The number of replicates (n) was three.

The response of the sampled benthic assemblage to the beyond-BACI design was analysed using permutational multivariate analysis of variance of linear models, based on distance measures (Anderson 2001; McArdle and Anderson 2001). In the case of the beyond-BACI design described here, each term in the model is balanced, allowing the linear model described to be used to construct complex analysis of variance (ANOVA) tables through a linear-regression-type approach by testing one term at a time under permutation (Anderson 2001; McArdle and Anderson 2001). This was done using the DISTLM version 4 procedure (Anderson 2004) through the construction of appropriate contrast matrices coding for the factors in the model (Legendre and Legendre 1998; Legendre and Anderson 1999). Contrast matrices for each term in the model and for the interactions between terms were created using the XMATRIX program (Anderson 2003).

To generate the distribution of the pseudo F statistic, unrestricted random permutations of raw data were used (Anderson and ter Braak 2003) and the p value for the test was also obtained using a random Monte Carlo sample from the theoretical asymptotic distribution of the pseudo F statistic under permutation (Legendre and Legendre 1998; Anderson and Robinson 2003).

In the case of this experimental design, particular interest lies in the interaction BI of factors 1 (B) and 3 (I) which, if significant, implies a long-lived press impact of hydraulic dredging; and in the interaction IT(B) of factors 2 (T(B)) and 3 (I) which, if significant, implies a short-lived pulse impact of hydraulic dredging (Underwood 1991, 1992). The logical null hypotheses being tested thus regarded the two interactions (BI = 0; IT(B) = 0). No test exists for factors 1 (B) and 3 (I) and their interaction (BI). The three tests were constructed by rearranging the linear combination of mean square estimates in the model to single out the variability due to the term of interest ($dtsnk_0^2$ for factor 1, $btsnk_i^2$ for factor 3, and $tsnk_{0i}^2$ for their interaction; see Table 1). Construction of the tests for each factor is summarized in Table 1. Whenever the terms of interest were significant, the nature of the impact was investigated by means of pairwise a posteriori comparisons using the DISTLM version 4 procedure (Anderson 2004), as described previously.

Seven groups of data were tested, in terms of both abundance and biomass: (1) the entire assemblage (excluding the target species), (2) the Mollusca (excluding the target species), (3) the Polychaeta, (4) the Crustacea, (5) detritivores, (6) suspensivores, and (7) predators and scavengers. A number of taxa were excluded from the trophic group analyses either because of lack of information or because of symbiotic and (or) parasitic life strategies; these were the bivalve *Montacuta ferruginosa*, the amphipods *Leucothoe serraticarpa* and *Microprotopus maculatus*; and anthozoans of the order Actiniaria.

Constrained ordination was used to visualize and analyse the data in the context of the specific a priori hypotheses of interest concerning differences among treatments by means of canonical analysis of principal components (CAP) (Anderson and Robinson 2003; Anderson and Willis 2003; Willis and Anderson 2003). The hypotheses were tested by obtaining a p value using permutation procedures (9999 permutations) on the canonical test statistics (squared canonical correlations, δ_i^2) generated by the analysis. The relative contribution of each taxon to the differences found was assessed using the correlation coefficient resulting between each taxon and the canonical axis in question.

All data were fourth-root transformed prior to analyses to balance the contribution of very abundant species and yet maintain intact information on relative abundances (Legendre and Legendre 1998). All analyses were carried out on similarity matrices based on the Bray–Curtis dissimilarity coeffi-

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Table 1. Mean square estimates for the balanced beyond-BACI design with an indication of the denominator mean squares used to calculate multivariate *F* ratios and the permutable units and number of permutations used for the test of each individual term and interaction.

Source of variation	Mean square estimates	df	Denominator mean squares	Permutable units	No. of permutations
B_j	$\delta_e^2 + n\delta^2_{I(B)S(I)} + dsn\delta^2_{I(B)} + dtsn\delta^2_B$	$(b-1)$	*F* ratio under permutation ($MSuP_B + MSuP_{BI(B)S} + MSuP_{T(B)}$)		
I_k	$\delta_e^2 + n\delta^2_{I(B)S(I)} + bsn\delta^2_{S(I)} + sn\delta^2_{IT(B)} + btsn\delta^2_I$	$(i-1)$	*F* ratio under permutation ($MSuP_I + MSuP_{BI(B)S} + MSuP_{IT(B)} + MSuP_{S(I)}$)		
BI_{jk}	$\delta_e^2 + n\delta^2_{I(B)S(I)} + sn\delta^2_{IT(B)} + tsn\delta^2_{BI}$	$(b-1)(i-1)$	*F* ratio under permutation ($MSuP_{BI} + MSuP_{BI(B)S} + MSuP_{IT(B)}$)		
$T(B)_{j(l)}$	$\delta_e^2 + n\delta^2_{I(B)S(I)} + sn\delta^2_{IT(B)}$	$b(t-1)$	$T(B)S(I)$	32 with 3 replicates	4999
$IT(B)_{kj(l)}$	$\delta_e^2 + n\delta^2_{I(B)S(I)} + sn\delta^2_{IT(B)}$	$b(t-1)(i-1)$	$T(B)S(I)$	32 with 3 replicates	4999
$S(I)_{m(k)}$	$\delta_e^2 + n\delta^2_{I(B)S(I)} + bsn\delta^2_{S(I)}$	$i(s-1)$	$T(B)S(I)$	32 with 3 replicates	4999
$BS(I)_{jm(k)}$	$\delta_e^2 + n\delta^2_{I(B)S(I)} + tn\delta^2_{BS(I)}$	$i(s-1)(b-1)$	$T(B)S(I)$	32 with 3 replicates	4999
$T(B)S(I)_{lj(mk)}$	$\delta_e^2 + n\delta^2_{I(B)S(I)}$	$bi(t-1)(s-1)$	Residuals	96	4999
$e_{n(lj(mk))}$	δ_e^2	$bits(n-1)$			

Note: X_{ijklmn}, datum for the nth replicate ($n = 1,\ldots,3$) in the mth site ($m = 1, 2$) in the lth time ($l = 1,\ldots,4$) in the kth impact ($k = 1, 2$); in the jth before–after ($j = 1, 2$); μ, population mean; B_j, jth level of before–after; I_k, kth level of impact; $T(B)_{j(l)}$, lth level of time nested within the jth level of before–after; $S(I)_{m(k)}$, mth level of site nested within the kth level of impact; BI_{jk}, $IT(B)_{kj(l)}$, $BS(I)_{jm(k)}$, and $T(B)S(I)_{lj(mk)}$ are interactions; $e_{n(lj(mk))}$, individual error associated with that replicate within that combination of treatments; b, i, t, and s are the number of levels in B, I, T, and S, respectively; n, number of replicates; df, degrees of freedom; MSuP, mean square under permutation.

cient, calculated to rank data into groups of similar entities (Legendre and Legendre 1998).

Species that had a correlation of $|r| > 0.20$ with the CAP canonical axes in question were tested for significant differences between treatments by means of univariate ANOVA tests and, where distribution assumptions were not met, by Kruskal–Wallis tests (Sokal and Rohlf 1995). No univariate analyses were carried out on species that occurred in less than three plots. Cochran's test was used to check for homogeneity of variances prior to ANOVA (Underwood 1997). Invoking the precautionary principle, the significance criterion for all tests was set at $\alpha_{[2]} = 0.10$ to minimize the probability of type-II errors, i.e., the probability of retaining the null hypothesis when it is false (Underwood 1997).

Results

Overall, 92 taxa were identified: 1 Cnidaria, 41 Mollusca, 27 Polychaeta, 19 Crustacea, 3 Echinodermata, and 1 Vertebrata. In terms of the number of species and wet weight, the Mollusca dominated the sampled community, while in terms of abundance, the population was dominated by the Polychaeta.

The entire macrobenthic assemblage sampled, i.e., the Polychaeta, the Crustacea, suspensivores, and detritivores, all exhibited small-scale variability (both spatial and temporal), with significant effects of nested random factors 2 (T) and (or) 4 (S) and (or) a significant interaction between the two, both in terms of abundance (Table 2) and biomass (Table 3). Such variability is expected when considering random factors, spatially because of the patchiness of soft-bottom infauna, and temporally because of natural phenomena such as recruitment. No effects, or impacts, of the experimental fishing tows were detected over and above this small-scale variability, neither pulse (significant IT(B) interaction) nor press (significant BI interaction).

Mollusca

Within the molluscan assemblage, the interaction BI between the two main factors (factors 1 (B) and 3 (I)) was significant upon consideration of both abundance and biomass data, allowing rejection of the null hypothesis of no impact (Tables 2 and 3). The differences in the molluscan assemblage from before to after were therefore different between the dredged sites and the control sites, and a sustained press impact of experimental hydraulic dredging was thus detected above natural variability. Molluscan biomass data (Table 3) reflected a less obvious response compared with abundance (Table 2), possibly attributable to a dominance of small-sized individuals overall.

Pairwise a posteriori comparisons done to compare levels of factor 1 (before versus after) at every single level of factor 3 (I), and levels of factor 3 (control versus dredged) at every single level of factor 1 on abundance data, revealed significant differences between control and dredged plots after experimental dredging only, while an intrinsic temporal difference within control and dredged plots was highlighted (Table 4). Thus an overall effect of experimental dredging was detected despite the temporal variability of the system, possibly caused by the fact that sampling was carried out in

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Table 2. Multivariate non-parametric analysis of variance carried out on the abundance of all groups tested.

Source of variation	Factor number	df	Entire community		Polychaeta		Crustacea		Mollusca		Detritivores		Suspensivores		Predators + Scavengers	
			MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC
B	1	1	0.5966	**0.0010**	0.4468	0.1770	0.4684	0.4160	0.7256	**0.0000**	0.4606	**0.0760**	0.4457	**0.0090**	0.7010	**0.0300**
T(B)	2	6	0.1730	**0.0002**	0.1944	**0.0002**	0.3275	**0.0444**	0.1477	**0.0002**	0.1725	**0.0002**	0.1183	**0.0002**	0.2023	**0.0014**
I	3	1	0.1930	0.5040	0.2561	0.2390	0.1255	0.9390	0.1665	0.1560	0.1810	0.5400	0.1258	0.1650	0.2851	0.5220
S(I)	4	2	0.1274	**0.0050**	0.1244	0.0640	0.3376	0.1006	0.0989	**0.0224**	0.1225	**0.0236**	0.0487	0.1408	0.2225	**0.0120**
BI	1 × 3	1	0.1444	0.2750	0.0918	0.7770	0.2912	0.5140	0.1579	**0.0240**	0.0713	0.8060	0.0777	0.4300	0.2165	0.3840
BS(I)	1 × 4	2	0.1133	**0.0148**	0.1556	**0.0194**	0.2883	0.1756	0.0585	0.2912	0.1297	**0.0154**	0.0646	**0.0366**	0.1815	**0.0442**
IT(B)	3 × 2	6	0.0616	0.4702	0.0612	0.5728	0.2047	0.3838	0.0465	0.5594	0.0469	0.6944	0.0390	0.2226	0.0989	0.3960
T(B)S(I)	2 × 4	12	0.0609	**0.0280**	0.0650	0.3928	0.1869	**0.0316**	0.0485	**0.0478**	0.0549	0.2806	0.0314	0.1158	0.0926	0.2114
Residuals		64	0.0485		0.0620		0.1296		0.0377		0.0496		0.0255		0.0808	
Total		95														

Note: B, factor 1 (before/after); T(B), factor 2 (time); I, factor 3 (impact); S(I), factor 4 (site); BI, IT(B), BS(I), and T(B)S(I) are interactions; *df*, degrees of freedom; MS, mean square; *p* MC, probability generated from Monte Carlo resampling.

Table 3. Multivariate non-parametric analysis of variance carried out on the biomass of all groups tested.

Source of variation	Factor number	df	Entire community		Polychaeta		Crustacea		Mollusca		Detritivores		Suspensivores		Predators + Scavengers	
			MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC	MS	*p* MC
B	1	1	0.5933	**0.0020**	0.8038	**0.0100**	0.3320	0.5930	0.5869	**0.0020**	0.6983	**0.0200**	0.2309	**0.0210**	0.5525	**0.0850**
T(B)	2	6	0.1897	**0.0002**	0.1890	**0.0002**	0.2812	0.1144	0.1843	**0.0002**	0.1925	**0.0002**	0.0847	**0.0002**	0.2270	**0.0006**
I	3	1	0.1982	0.5420	0.2300	0.3000	0.0661	0.9980	0.1541	0.3450	0.2300	0.4750	0.0795	0.1100	0.2291	0.7680
S(I)	4	2	0.1487	**0.0020**	0.1618	**0.0064**	0.5440	**0.0120**	0.1141	**0.0426**	0.1348	**0.0098**	0.0350	0.4884	0.2922	**0.0026**
BI	1×3	1	0.1938	0.1530	0.1102	0.6650	0.2300	0.6970	0.1489	**0.0880**	0.2497	0.1140	0.0669	0.1660	0.2606	0.4100
BS(I)	1×4	2	0.1268	**0.0080**	0.1311	**0.0222**	0.3109	0.1394	0.0829	0.2308	0.1503	**0.0038**	0.0433	0.2956	0.1827	**0.0660**
IT(B)	3×2	6	0.0743	0.2524	0.0678	0.2556	0.2239	0.2872	0.0603	0.6262	0.0496	0.6330	0.0312	0.6964	0.1580	**0.0538**
T(B)S(I)	2×4	12	0.0657	0.1828	0.0557	0.2118	0.1861	**0.0794**	0.0650	0.2326	0.0546	0.1986	0.0359	**0.0202**	0.1038	0.7786
Residuals		64	0.0592		0.0481		0.1398		0.0587		0.0474		0.0261		0.1200	
Total		95														

Note: B, factor 1 (before/after); T(B), factor 2 (time); I, factor 3 (impact); S(I), factor 4 (site); BI, IT(B), BS(I), and T(B)S(I) are interactions; *df*, degrees of freedom; MS, mean square; *p* MC, probability generated from Monte Carlo resampling.

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Table 4. Pairwise a posteriori contrasts comparing levels of factor 3 at each single level of factor 1, and levels of factor 1 at each single level of factor 3 for the abundance of molluscan species.

	Comparison	*p*MC
Abundance		
Level of factor 1 (before/after)		
Before	Control versus dredged	0.300
After	Control versus dredged	0.014
Level of factor 3 (impact)		
Control	Before versus after	0.000
Dredged	Before versus after	0.016
Biomass		
Level of factor 1 (before/after)		
Before	Control versus dredged	0.435
After	Control versus dredged	0.097
Level of factor 3 (impact)		
Control	Before versus after	0.001
Dredged	Before versus after	0.103

Note: *p*MC, probability generated from Monte Carlo resampling.

a period corresponding to the recruitment and settlement of many molluscan species.

In terms of biomass data, the results of the a posteriori comparisons revealed significant differences between control and dredged plots only after experimental dredging, but temporal differences were of some importance within control plots alone (Table 4). This result may be caused by the possible dominance of recruits of many species or by small-sized species whose removal, in terms of biomass, could be insignificant but whose growth and mere presence could significantly increase variability in control plots, especially in light of the patchy dispersion associated with assemblages of juvenile molluscs.

The CAP analyses carried out separately on the main effects (B and I) confirmed the multivariate non-parametric analysis of variance results (Table 5). The canonical axes corresponding to the main effects, when plotted against each other, showed the interaction in two-dimensional space (exemplified for abundance in Fig. 2), where a separation of the molluscan assemblage into before and after dredging, and into dredged and control was evident. While the dispersion of points from before to after and from dredged to control was not dramatically different, the direction of change of before/control (BC) to after/control (AC) was in a different direction from that of before/dredged (BD) to after/dredged (AD; Fig. 2).

The relative distinctiveness of assemblages in the various treatments was further confirmed by the high leave-one-out allocation success percentages (Table 5), although reallocation success in levels of factor 3 (I) was lower than that for factor 1 (B), indicating a relatively greater difficulty in the separation of dredged and control plots.

The correlations of individual species ($|r| > 0.20$) with the CAP axes for B and I showed that, in terms of abundance (Fig. 3), three species increased after dredging in the dredged plots. These were the bivalves *M. ferruginosa* and *Loripes lacteus* and the gastropod *Nassarius reticulatus*. By contrast, three bivalve species decreased in dredged plots after dredging. These were *Phaxas pellucidus*, *A. alba*, and *Corbula gibba*. Finally, the bivalve *Lentidium mediterraneum* decreased after dredging in control plots. Univariate ANOVA (F) or Kruskal–Wallis (H) test results indicated no significant differences between before and after for *M. ferruginosa* and *L. lacteus*, while significant differences were found for *N. reticulatus*, which increased, and *P. pellucidus*, *C. gibba*, and *A. alba*, which all decreased after dredging (Fig. 4).

Similar results were obtained upon analysis of biomass data but with subtle differences. *Loripes lacteus* no longer characterized the mollusc assemblage found in dredged plots after dredging, neither did the bivalve *P. pellucidus*, which dwindled in importance with respect to those species denoting a decrease within dredged plots after dredging. The bivalve *Pharus legumen* and the gastropod *Acteon tornatilis* both increased in dredged plots after dredging. Univariate Kruskal–Wallis tests carried out on the biomass of each species indicated a significant difference between before and after in dredged plots for *A. alba* alone ($H = 11.575$, $p = 0.0007$).

Predators and scavengers

The results obtained on analysis of predator and scavenger abundance data highlighted a significant variability at the small scale with no pulse or press impacts of hydraulic dredging being detectable above this. Biomass data, however, told a different story. The interaction IT(B) between factors 2 (T) and 3 (I) was significant in terms of biomass, meaning that there were significant differences between dredged and control plots that varied differently among sampling times. This denotes a pulse impact of hydraulic dredging with respect to the predator and scavenger assemblage whose multivariate location (or variance) increased or decreased at a specific time to recover soon after. The pairwise a posteriori comparisons revealed significant differences in the scavenger and predator assemblage between control and dredged plots at time "after 1" only, i.e., on the first sampling date after the experimental impact had occurred, and such differences were no longer discernible subsequently (Table 6).

CAP analysis carried out on time "after 1" alone revealed the high allocation success in both groups (control and dredged) and the percentage of variance explained by the choice of m principal coordinate axes underlined the high relative distinctiveness in dredged and control plots (Table 7).

The main taxa responsible for the difference between control and dredged sites at "time 1 after" (A1) the impact were identified upon examination of the correlation between each taxon and the CAP axis for A1, and the results are summarized in Table 8. Three polychaete taxa (*Sigalion mathildae*, *Nephthys* sp., and *Phyllodoce* sp.), three crustacean species (*Philocheras monacanthus*, *Processa modica*, and *Liocarcinus vernalis*), and one molluscan species (*Nassarius pygmaeus*) were associated with control sites, while two polychaete taxa (*Lumbrineris* sp. and Maldanidae) and three molluscan species (*Bela nebula*, *Philine aperta*, and *Nassarius mutabilis*) were associated with the dredged plots. Differences between dredged and control plots for each species were investigated by means of univariate Kruskal–Wallis tests, and the results (Fig. 5) indicated a significant increase of *S. mathildae* in

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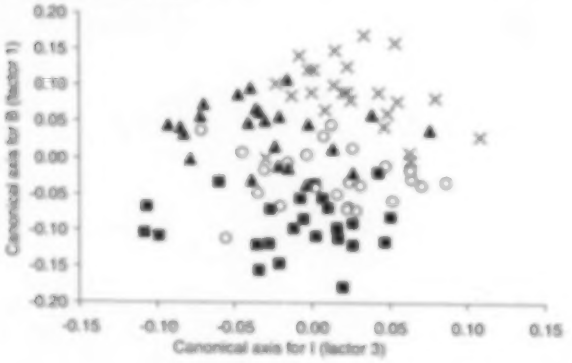
Table 5. Results of the canonical analysis of principal coordinates examining the effects of factors 1 (before/after) and 3 (impact) on the molluscan soft-sediment assemblage in terms of abundance and biomass.

				Allocation success (%)				
	Factor	*m*	% Var.	Level	Level	Total	δ^2	*p*
Abundance	B	7	80.894	87.500 (before)	83.333 (after)	85.417	0.603	0.0002
	I	7	80.894	62.500 (dredged)	64.583 (control)	63.542	0.196	0.0054
Biomass	B	5	60.066	77.083 (before)	87.500 (after)	82.292	0.512	0.0002
	I	11	95.909	58.333 (dredged)	72.917 (control)	65.625	0.236	0.0140

Note: % Var., percentage of variance explained by the choice *m* of principal coordinate axes; allocation success, percentage of points correctly allocated in each level; δ^2, canonical squared correlation.

Fig. 2. Two-dimensional scatter plot of the canonical axes for factor 1 (before/after) and factor 3 (impact) using molluscan abundance data. ■, before/dredged; ○, before/control; ▲, after/dredged; ×, after/control.



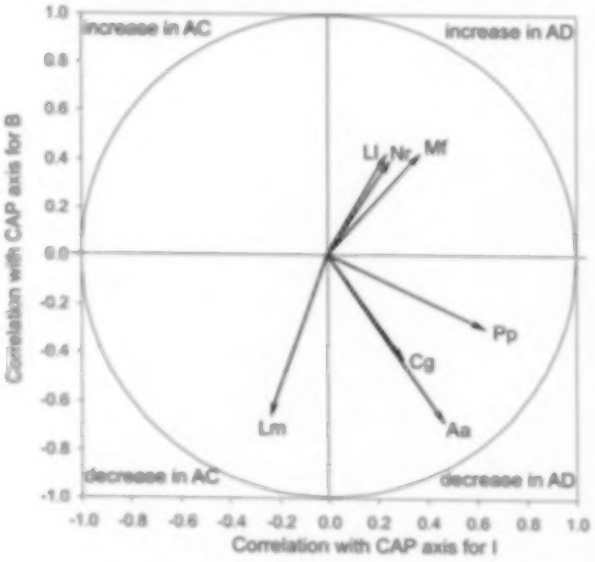
control plots and a significant increase of *B. nebula* in dredged plots. An increase in the mean biomass of *N. mutabilis* and *P. aperta* was evident within dredged plots but was not statistically significant.

Discussion

The results of this impact study highlighted the overall dominance of the community by polychaetes in terms of abundance. The relative numerical dominance of polychaetes implies that they are well established, and this may be a result of the sustained fishing pressure to which the area has been subjected for over 30 years. Dominance of small short-lived opportunist species (*r* strategists), such as polychaetes, to the detriment of larger bodied, longer lived species (*k* strategists), such as molluscs, is widely reported in literature to be a consequence of chronic long-term anthropogenic disturbance, including fishing (Pearson and Rosenberg 1978; Warwick 1986; Kaiser et al. 2002).

Upon multivariate analysis of the entire sampled macrozoobenthic assemblage, the Polychaeta, the Crustacea, and also detritivorous and suspensivorous guilds, no impact or effect of the experimental fishing tows was discernible over natural variability. In contrast, the results obtained by Currie and Parry (1996) showed that effects of scallop dredging were discernible for the Polychaeta, the Mollusca, and for deposit feeders. The lack of response of these groups of taxa reported here could indicate a possible historical effect of fishing, in that an altered stable state or a persistent situation of moderate disturbance has become the norm, rendering the

Fig. 3. Correlations of individual molluscan species (|r| > 0.20), in terms of abundance, with the canonical analysis of principal coordinates axes for factor 1 (before/after) and factor 3 (impact) of Fig. 2. Aa, *Abra alba*; Cg, *Corbula gibba*; Ll, *Loripes lacteus*; Lm, *Lentidium mediterraneum*; Mf, *Montacuta ferruginosa*; Nr, *Nassarius reticulatus*; Pp, *Phaxas pellucidus*; AD, dredged plots after dredging; AC, control plots after dredging. The length of arrows is proportional to the correlation with the two axes.



detection of any further impact impossible (Currie and Parry 1996; Bradshaw et al. 2000; Hall-Spencer and Moore 2000). Collie et al. (2000) showed that, in sandy sediments, benthic communities recovered within approximately 100 days, implying that two or three disturbance events per year would be a threshold level of intensity within which the structure of the community would not be significantly changed. Thus, fishing activity of an intensity greater than this could be enough to maintain the community in a condition of permanent alteration. The results of a study on the use of GPS-based data loggers as a tool for stock assessment carried out in the study area pertinent to this investigation revealed that, within a 6-month period, each 50 m × 50 m square examined was disturbed an average of 6.4 times by a subsample of the fleet (17 vessels out of 73; Marrs et al. 2002). It is therefore most likely that the macrobenthic community examined would be in a permanently altered condition due to

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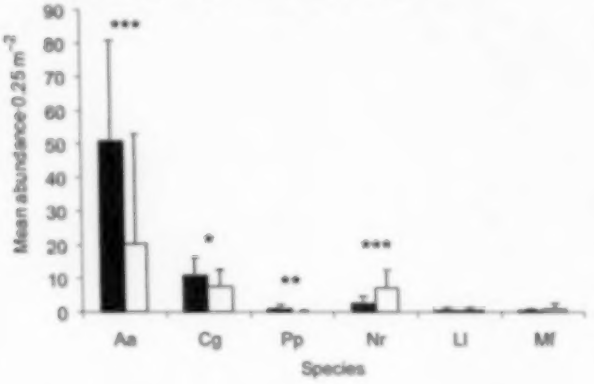
Fig. 4. Mean abundance (+SD) of molluscan species represented in Fig. 3, averaged across dredged sites before (BD; solid bars) and after (AD; open bars) dredging, with an indication of analysis of variance (F) or Kruskal–Wallis (H) test results for the comparison between BD and AD for each species. Aa, *Abra alba* ($F = 11.42$); Cg, *Corbula gibba* ($F = 3.62$); Pp, *Phaxas pellucidus* ($H = 5.465$); Nr, *Nassarius reticulatus* ($F = 11.94$); Ll, *Loripes lacteus* ($F = 0.14$); Mf, *Montacuta ferruginosa* ($F = 1.20$); °, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$.



the intense hydraulic dredging activity in the area. This was confirmed by the high abundance of taxa typical of disturbed sediments (e.g., *Owenia fusiformis* and *Ampelisca* sp.) or moderately disturbed transitional zones (e.g., *A. alba* and *C. gibba*; see Pearson and Rosenberg 1978; Newell et al. 1998). Indeed, a shortcoming of most fisheries impact studies is that they are done in environments where fishing activities have been going on for centuries and data regarding the prefishing status of any benthic community are sparse or unsuitable for the unequivocal attribution of community shifts to fishing (Hill et al. 1999; Collie et al. 2000; Frid et al. 2000).

The lack of response of the suspensivore assemblage was surprising. This portion of the benthic community is generally considered to be sensitive, in that the resuspension of fine particles by hydraulic dredging may contribute to the clogging of their filtering organs (Pranovi and Giovanardi 1994; Newell et al. 1998; Kaiser et al. 2002). This lack of response may be due to an overall adaptation of the assemblage to repeated disturbance (Newell et al. 1998). In addition, the behaviour of the cloud of sediment resuspended by hydraulic dredges in the study area was found to be variable, short lived, and heavily dependent on the presence of bottom currents (Hauton et al. 2002). Water current conditions on the day of experimental dredging may have been favourable to the efficient removal of the cloud of sediment or, more likely, the amount of sediment resuspended by a single experimental tow (0.001–0.07 $g \cdot L^{-1}$; Hauton et al. 2002) was insufficient to create any negative response in the suspensivourous assemblage in question.

Gambi et al. (1982) found the molluscan assemblage to be the most efficacious descriptor of the benthic communities inhabiting unconsolidated sediments between depths of 3 and 100 m. In our study, a consistent ongoing press impact of hydraulic dredging on the molluscan assemblage was revealed upon consideration of both abundance and biomass

Table 6. Pairwise a posteriori contrasts comparing levels of factor 3 at each single level of factor 2 for the biomass of scavenger and predator species.

Level of factor 2 (time)	Comparison	pMC
Before 1	Control versus dredged	0.149
Before 2	Control versus dredged	0.581
Before 3	Control versus dredged	0.612
Before 4	Control versus dredged	0.609
After 1	Control versus dredged	0.009
After 2	Control versus dredged	0.324
After 3	Control versus dredged	0.200
After 4	Control versus dredged	0.645

Note: pMC, probability generated from Monte Carlo resampling.

data. The inclusion of temporal and spatial replication within the design ruled out the possibility that such differences could have been due simply to the fact that samples were taken at two different times or in two different places (Hurlbert 1984; Underwood 1991, 1994). The results obtained from abundance and biomass data were slightly different. A posteriori comparisons of abundance data indicated there were differences between dredged and control plots only after the experimental tows, and differences between before and after the impact in both dredged and control sites. Biomass data still indicated differences between dredged and control sites only after dredging, but with temporal differences being of some importance in control sites only. This result may be linked to the significant removal of larger bodied species in dredged plots, indicating that dredging induces a reduction in the temporal variability of biomass within the disturbed system. Underwood (1992, 1994) and Warwick and Clarke (1993) suggested postimpact changes in spatial and temporal variability might be more sensitive indicators of stress and disturbance. Lindegarth et al. (2000) reported increased small-scale spatial and temporal variability in soft-sediment benthic assemblages as a response to shrimp trawling, but also underlined that the direction of change in variability from before to after the disturbance is dependent on the homo- or hetero-geneity of the system before dredging.

Numerically, an overall increase of the bivalves *M. ferruginosa* and *L. lacteus* and the gastropod *N. reticulatus*, and an overall decrease of the bivalves *C. gibba*, *P. pellucidus*, and *A. alba* were found to have occurred in dredged plots after experimental dredging. Significant differences between before and after within dredged plots were revealed for *N. reticulatus*, *C. gibba*, *P. pellucidus*, and *A. alba* only. Similar results were obtained upon analysis of biomass data but with subtle differences (due to small size, e.g., *L. lacteus* or light-weight shells, e.g., *P. pellucidus*). The bivalve *P. legumen* and the gastropod *A. tornatilis* were both found to increase in dredged areas after dredging in terms of biomass. Among all species, the only one in which biomass significantly changed in dredged plots after dredging was *A. alba*, which decreased.

The netted dogwhelk *N. reticulatus* increased significantly in dredged plots after dredging. *Nassarius reticulatus* is a scavenger able to detect and locate food sources in the form of carrion very rapidly (Davenport and Moore 2002) and was most probably attracted by the large amounts of the car-

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Table 7. Results of the canonical analysis of principal coordinates examining the effects of level "after 1" of factor 2 (time (before/after)) on the biomass of the predator and scavenger assemblage.

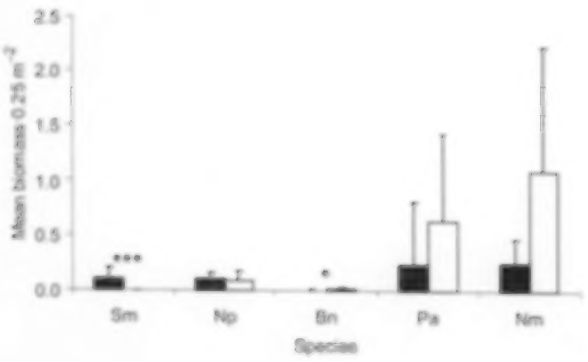
			Allocation success (%)				
Level compared	*m*	% Var.	Level	Level	Total	δ^2	*p*
A1	5	91.7	100 (D)	100 (C)	100	0.592	0.0014

Note: % Var., percentage of variance explained by the choice *m* of principal coordinate axes; allocation success, percentage of points correctly allocated in each level; δ^2, canonical squared correlation; A1, after 1; D, dredged; C, control.

Table 8. Correlation coefficients for individual taxa ($|r| > 0.20$) with the canonical axis for level A1 of factor 2 (time), where a positive correlation indicates an association with control sites and a negative correlation indicates an association with dredged sites.

Positive correlation (control)		Negative correlation (dredged)	
Sigalion mathildae	0.9048	*Bela nebula*	–0.5230
Nemertea	0.4619	*Philine aperta*	–0.4759
Nephtys sp.	0.4348	*Nassarius mutabilis*	–0.3530
Philocheras monacanthus	0.4348	*Lumbrineris* sp.	–0.3070
Processa modica	0.3650	Maldanidae	–0.2084
Liocarcinus vernalis	0.2825		
Nassarius pygmaeus	0.2805		
Phyllodoce sp.	0.2528		

Fig. 5. Mean biomass (g; +SD) of predator and scavenger species averaged across control (C; solid bars) and dredged (D; open bars) sites, with an indication of Kruskal–Wallis (*H*) test results for the comparison of C and D for each species. Sm, *Sigalion mathildae* ($H = 9.4661$); Np, *Nassarius pygmaeus* ($H = 0.2308$); Bn, *Bela nebula* ($H = 3.1884$); Pa, *Philine aperta* ($H = 2.1064$); Nm, *Nassarius mutabilis* ($H = 0.7784$); *, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$.



rion generated by hydraulic dredging. The damage inflicted by trawling on the epibenthos of a subtidal habitat in Hong Kong has been suggested as the cause for the distinct dominance by another nassariid gastropod, *N. siquijorensis* (Britton and Morton 1994).

Regarding species that decreased in the dredged plots after dredging, the shells of *P. pellucidus* and *A. alba* are extremely fragile, rendering them very vulnerable when collected with hydraulic dredges and subsequent sieving operations. Bergman and van Santbrink (2000) reported both species among the most vulnerable to beam trawling in the Dutch North Sea owing to their fragility. Piet et al. (2000) reported similarly for *A. alba*. In contrast, MacDonald et al. (1996) categorized *A. alba* as having an intermediate sensitivity and did not include it in a list of possible indicator species; similar results were reported by Collie et al. (2000). *Abra alba* is an important component of the benthic community within the study area and the high significance of the results obtained point to this fragile species as a candidate indicator species for the effects of hydraulic dredging on the benthic community resident in the study area. This was confirmed by a larger scale study aimed at evaluating the effects of differing levels of fishing intensity on the benthic community (E.B. Morello, C. Froglia, R.J.A. Atkinson, and P.G. Moore, unpublished data).

Corbula gibba, on the other hand, is an extremely resilient species typical of transitional environments and enriched sediments (Pérès and Picard 1964; Pearson and Rosenberg 1978) and its thick, sturdy shell renders direct physical damage by dredging unlikely (Rumohr and Krost 1991; Morton 1996). Bergman and van Santbrink (2000) reported very low mortalities due to beam trawling and included this species among the most resistant, as did Collie et al. (2000). Pranovi and Giovanardi (1994) underlined its resilience rather than resistance to hydraulic dredging in the Venetian lagoon. Tuck et al. (1998) indicated that *C. gibba* was among the most vulnerable species with respect to bottom trawling, and Currie and Parry (1996) found the abundance of the congeneric *C. coxi* to be significantly negatively affected by dredging in the long term. Hill et al. (1999) found the historical differences in the Irish Sea benthic communities to be due to the high densities of *C. gibba* in the past. *Corbula gibba* is restricted to the most superficial layer of the sediment (Moodley et al. 1998). Hydraulic dredging removed very high proportions of the population. This implies that under normal commercial fishing conditions, when over half of the fleet may be operating within the same area, considerable amounts of *C. gibba* will be relocated to different patches. The effects of this are unknown but worthy of further investigation. *Corbula gibba* tends to form dense mats within the first centimetre of sediment (E.B. Morello, per-

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sonal observation) and the implications of the addition of more individuals on top of those already established may be deleterious with respect to the redox conditions of the sediment.

Marine fishing activities may have two contrasting effects on scavengers. They may lead to their reduction by removing them from the sea or they may enhance their success by providing them with food, and the latter effect is thought to be the pre-eminent one (Britton and Morton 1994).

Analysis of the predator and scavenger assemblage indicated a short-lived pulse impact of hydraulic dredging on this assemblage with significant differences between dredged and control areas only on the first day of sampling immediately after the fishing disturbance events. The short-lived nature of the scavenger response has been widely reported in literature (Kaiser and Spencer 1996; Ramsay et al. 1997; Bergmann et al. 2002).

Identification of the taxa driving these differences revealed the particular importance of the polychaete *S. mathildae*, which was very highly correlated with control sites. Craeymeersch et al. (2000) found varying responses of *S. mathildae* to trawling: it was found to increase within fished areas offshore but was correlated with undisturbed areas further inshore. *Sigalion mathildae* is a deep burrower living between 15 and 20 cm below the sediment surface (Craeymeersch et al. 2000) and thus should not be particularly vulnerable to hydraulic dredging, which does not dig deeper than 5–6 cm. Its high correlation with control areas, and thus its relatively low biomass within dredged areas, may be due to the fact that dredging contributed by exposing it to increased predation by displacing surface sediment.

Three scavenging gastropod species, namely *B. nebula*, *N. mutabilis*, and *P. aperta*, increased markedly in dredged sites, but differences between dredged and control plots were statistically significant for the former species only. Increases in scavenger abundances as a result of increased food availability in fished areas are widely reported in literature (Wassenberg and Hill 1987; Ramsay et al. 1997, 1998). *Nassarius mutabilis* is one of the most abundant species within the fine well-sorted sand community in the examined area and the target of a locally important creel fishery. It is also an active scavenger able to rapidly locate food from a distance (Bedulli 1976; Crisp 1978). Despite the strong difference in its biomass between dredged and control plots, this difference was not significant. Another nassariid gastropod is very abundant within the same area, namely *N. reticulatus*. Its feeding habits and biology are very similar to those exhibited by *N. mutabilis*, but a press impact on *N. reticulatus* was revealed upon analysis of the molluscan assemblage. Differences in response to fishing between ecologically similar congeneric species have been reported for two hermit crab species (Ramsay et al. 1996) and competitive interactions were sought as an explanation for such differences. This is unlikely in the case of the two *Nassarius* species in question. The fact that one species, *N. mutabilis*, is targeted by a conspicuous local commercial fishery and the other is not is more likely to account for such differences.

Studies regarding the impacts of fishing activities are burdened with numerous difficulties. Among these are the fact that historically unfished areas are seldom available for comparison and that the vast majority of benthic species have multiple-stage life cycles that render the influence of factors extrinsic to disturbance (i.e., factors which will control recruitment and settlement) of utmost importance in controlling community dynamics. In addition to this is the fact that the effects of fishing activities on newly settled organisms are unknown. The overall effects of fishing will thus depend not only on the spatial and temporal scales of fishing disturbance, but also on the life histories of each single species within the community, interspecific interactions, and the interactions between them and the environment (Langton and Auster 1999; Bradshaw et al. 2000). Furthermore, in sand communities, the recovery of population-level characteristics (e.g., size structures) after disturbance was found to be delayed with respect to the recovery of the community in its entirety, thus resulting in complex succession stages enhancing the existing heterogeneity and further diminishing the predictability of both impact and recovery dynamics (Zajac and Whitlatch 2003). The heterogeneity of fishing disturbance and the unpredictability of natural disturbance events, such as storms, which are of great importance within shallow sandy habitats, further decrease our power to predict recovery patterns and pathways. Despite this, the results reported here highlight important aspects of short-term, and possibly medium-term, effects of hydraulic dredging within the study area examined which had not previously been documented. Most importantly, the fact that, despite intensive fishing that has been going on for decades and a benthic community that is typical of a moderately disturbed environment, the effects of fishing on community structure were still discernible above natural variation. This was particularly true for sensitive taxa such as those belonging to Mollusca. Despite this, identification of acute impacts through small-scale studies is not enough and may not reflect the chronic effects induced by an entire fleet (Collie et al. 2000; Kaiser et al. 2000; Schratzberger et al. 2002). Further studies need to be carried out on a larger scale and in a manner that will unambiguously identify the response of the community to different levels of fishing intensity, in an attempt to establish a threshold intensity applicable to the community in question and thus provide tools for the correct management of the fishery. One possible way to carry out such studies could be through manipulation of the existing commercial fleet and the establishment of an experimental rotation of areas. In addition, greater importance should be given to the effects of fishing activities on different life-cycle stages of the species present, with particular reference to newly settled individuals. Optimistically, such a strategy would give managing bodies a biological tool to be used in the establishment of closure areas and periods as an alternative, or a supplement, to the economic and political tools used at present. However, this would imply the complete cooperation of the fleet and stakeholders, a condition that may be unrealistic for reasons related to the high short-term economic returns yielded by the management strategies used at present.

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The influence of habitat complexity on littoral invertebrate distributions: patterns differ in shallow prairie lakes with and without fish

Michael D. Rennie and Leland J. Jackson

Abstract: Relationships between littoral habitat complexity and invertebrate distributions in fishless lakes are not well understood compared with well-documented relationships in lakes with fish. We examined littoral invertebrate distributions over fine-scale gradients of weed-bed habitat complexity and contrasted these patterns in four shallow prairie lakes — two with fish and two without. The above-sediment portion of submerged macrophytes and associated invertebrates was sampled from three littoral microhabitats: weed-bed centres (highly complex), weed-bed edges (moderately complex), and single plants that grew apart from distinct weed beds (least complex). Total invertebrate densities in fishless lakes did not differ between littoral microhabitats, nor were they correlated with macrophyte biomass. In contrast, total invertebrate densities in lakes with fish increased with microhabitat complexity and were positively correlated with macrophyte biomass. Weed-bed complexity also affected littoral invertebrate community structure; in all lakes, the proportion of filter-feeders decreased with increasing microhabitat complexity, but the proportion of predatory invertebrates was greater overall in fishless lakes than in lakes with fish. Our results demonstrate that small-scale variation in littoral microhabitat complexity can lead to specific patterns of invertebrate distribution that systematically differ between lakes with and without fish, and that these systematic differences may be mediated through top-down mechanisms.

Résumé : Les relations entre la complexité de l'habitat littoral et la répartition des invertébrés dans les lacs sans poissons sont mal comprises, alors qu'elles sont bien étudiées dans les lacs qui ont des poissons. Nous avons examiné les répartitions des invertébrés littoraux le long de gradients à échelle fine de complexité de l'habitat des herbiers et comparé ces patrons dans quatre lacs peu profonds des prairies, deux avec des poissons et deux sans poissons. Nous avons échantillonné les macrophytes et les invertébrés associés dans la partie supérieure aux sédiments dans trois microhabitats littoraux, soit le centre des herbiers (très complexe), le bord des herbiers (moyennement complexe) et les plantes qui poussent isolément de tout herbier défini (le moins complexe). Les densités totales d'invertébrés dans les lacs sans poissons ne diffèrent pas d'un microhabitat littoral à un autre et elles ne sont pas corrélées à la biomasse des macrophytes. En revanche, la biomasse totale des invertébrés dans les lacs avec poissons augmente en fonction de la complexité du microhabitat et est en corrélation positive avec la biomasse des macrophytes. La complexité des herbiers affecte aussi la structure des communautés d'invertébrés : dans tous les lacs, la proportion des organismes filtreurs décroît à mesure que la complexité des microhabitats augmente; globalement, la proportion d'invertébrés prédateurs est plus grande dans les lacs sans poissons que dans les lacs avec poissons. Nos résultats démontrent que la variation à petite échelle de la complexité des microhabitats littoraux peut entraîner l'établissement de patrons spécifiques de répartition des invertébrés qui diffèrent systématiquement dans les lacs avec et sans poissons; ces différences systématiques s'expliquent peut-être par les mécanismes à action descendante.

[Traduit par la Rédaction]

Introduction

The relationship between ecosystem structure and function is a fundamental issue in ecology. The variety of resources available to organisms (e.g., food, nesting sites, and refugia) is dictated by the physical structure and complexity of their habitats. Compared with nonvegetated areas of lakes, sections of the littoral zone that harbour aquatic macrophytes are structurally more complex and typically support a greater abundance, biomass, and diversity of macroinvertebrates (Pardue and Webb 1985; Beckett et al. 1992; Blindow et al. 1993). Traditional sampling techniques have assumed

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Table 1. Physical and chemical attributes of lakes near Strathmore, Alberta, Canada.

	Dawson Lake	Mushroom Lake	SGC	SPA
Secchi depth (m)	1.4	1.2	1	1.3
Turbidity (NTU)[a]	3.49 ± 0.68	2.32 ± 0.14	7.70 ±0.27	7.57 ± 0.58
Total phosporus ($\mu g \cdot L^{-1}$)[a]	937.3 ± 391.0	547.7 ± 468.0	67.36 ± 33.39	75.66 ± 16.44
DOC ($mg \cdot L^{-1}$)[a]	31.12 ± 2.42	26.05 ± 6.34	11.72 ± 2.18	23.48 ± 11.79
pH[a]	9.44 ± 0.25	9.01 ± 0.55	8.71 ± 0.21	8.26 ± 0.17
Conductivity ($mS \cdot cm^{-1}$)[a]	3.937 ± 0.006	0.597 ± 0.030	0.558 ± 0.006	0.732 ± 0.004
Temperature (°C)[a]	22.5 ± 0.8	22.2 ± 0.7	20.3 ± 0.8	20.0 ± 1.5
Area (km^2)	0.314	0.117	0.103	0.02
Maximum depth (m)	1.7	1.3	1.3	3.3

Note: SGC, Strathmore Golf Course; SPA, Strathmore Picnic Area; NTU, nephelometric turbidity units; DOC, dissolved organic carbon.

[a]Values are given as the mean ± standard deviation and are summer averages from June, July, and August samples.

plant density and invertebrate distributions within a weed bed to be homogeneous (Cyr and Downing 1988*a*, 1988*b*; Zimmer et al. 2000). Realistically, variability in growth form and density of plants likely contributes to significant structural heterogeneity within weed beds. The density and complexity of weed beds can directly interfere with the foraging efficiency of both fish and invertebrate predators (Crowder and Cooper 1982; Tokeshi and Pinder 1985; Dibble et al. 1996). Therefore, fine-scale habitat complexity (within weed beds) may directly contribute to differences in the distribution and abundance of littoral invertebrates and thus the flow of energy through littoral food webs (Lillie and Budd 1992).

Previous research in lakes with fish has shown that total invertebrate abundance, biomass, and diversity are strongly influenced by a variety of measures describing macrophyte habitat complexity. These include the type and number of plant species present (Keast 1984; Dionne and Folt 1991), the degree of substrate dissectedness (Hacker and Steneck 1990; Kershner and Lodge 1990; Jeffries 1993), plant biomass (Downing 1986; Cyr and Downing 1988*a*; Paterson 1993), plant biomass density (Duarte and Kalff 1990), plant surface area (Brown et al. 1988; Taniguchi et al. 2003), and the complexity of the immediate surrounding environment (Sloey et al. 1997). In contrast to this relatively large body of evidence, the effect of macrophyte habitat complexity — according to any of the measures described above — on invertebrate communities in fishless lakes remains unknown.

Many fish species preferentially forage among aquatic weed beds (Mittlebach 1981; Tompkins and Gee 1983; Trebitz et al. 1997). The primary mode of prey detection by many littoral fish is visual (Croy and Hughes 1991). Thus, it is likely that fish play an important role in shaping observed relationships between macrophytes and littoral invertebrate assemblages. Because fish feed most efficiently where they can best see and access prey (Crowder and Cooper 1982; Engel 1988; Manatunge et al. 2000), negative effects of predation by fish on littoral invertebrate communities should be most pronounced in less complex habitats such as low-density weed beds or along the edges of dense weed beds. Very dense patches of macrophytes can reduce both foraging success and efficiency of littoral fish (Dibble et al. 1996). Foraging preferences of fish could in turn affect preferential feeding habitats of invertebrate predators, either through predation (by fish on predatory invertebrates) or through competition for shared prey, resulting in trophic cascades or more complex trophic interactions through intraguild predation (Hart 2002). Thus, the effects of fish predation on littoral invertebrate abundance and community structure likely vary over small spatial scales in aquatic weed beds and may be correlated with fine-scale gradients of weed-bed structural complexity.

Our objective was to examine the degree to which the structure of aquatic weed beds affect their function as invertebrate habitat across the range of macrophyte structural complexity typically encountered in shallow prairie lakes, while examining the role that fish presence or absence might play in shaping these relationships. We selected three operational levels of habitat complexity over which to compare invertebrate assemblages — individual plants, weed-bed edges, and weed-bed centres — and contrasted these patterns in two lakes with fish and two lakes without fish. This design allowed us to examine in detail littoral invertebrate distributions across gradients of microhabitat complexity within a few lakes with contrasting food-web structure, as opposed to comparing habitats of a single type over a greater number of lakes. Previous studies have examined broad-scale patterns of invertebrate distributions across lakes with and without fish (Zimmer et al. 2000). Because predator impacts in open ecosystem experiments have been shown to be scale-dependent (Englund et al. 2001), our sampling design was purposely chosen to assess littoral invertebrate distributions across gradients of macrophyte complexity smaller than those typically considered in lake studies. This allowed us to investigate differences at spatial scales relevant to the fish and invertebrates occupying these habitats. To assess the generality of our findings, we compared our results from lakes with fish with published patterns observed elsewhere.

Materials and methods

Site description

During the summer of 1998 we sampled four shallow prairie lakes in and around the town of Strathmore, Alberta, Canada (51°2.5′N, 113°24.5′W). The lakes in Strathmore Picnic Area (SPA) and Strathmore Golf Course (SGC) both contained naturally occurring brook stickleback (*Culaea inconstans*). SPA is stocked annually with rainbow trout (*Oncorhynchus mykiss*) by the town of Strathmore. Mushroom Lake and Dawson Lake were deemed fishless after random transects were swum with SCUBA once a month

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Table 2. Food-web characterization of the lakes under study.

	Dawson Lake	Mushroom Lake	SGC	SPA
Dissolved phosphorus concentration ($\mu g \cdot L^{-1}$)[a]	928 ± 348	338 ± 198	20.5 ± 7.3	35.4 ± 14.7
Chlorophyll *a* concentration ($\mu g \cdot L^{-1}$)[a]	0.5 ± 0.18	2.57 ± 1.54	0.35 ± 0.06	2.89 ± 3.62
Total macrophyte biomass ($kg \cdot m^{-2}$)	11.80	2.05	5.78	6.98
Invertebrate abundance ($no. \cdot m^{-2}$)[a]				
Weed-bed centre	51 734 ± 7 466	31 789 ± 8 123	95 066 ± 18 105	287 150 ± 64 548
Weedbed edge	54 700 ± 11 906	34 201 ± 7 374	46 203 ± 9 380	72 161 ± 23 513
Individual plants	36 253 ± 6 060	31 557 ± 8 029	23 901 ± 13 649	12 622 ± 3 078
Fish species present	Fishless	Fishless	*Culaea inconstans*	*Oncorhynchus mykiss*, *C. inconstans*

Note: SGC, Strathmore Golf Course; SPA, Strathmore Picnic Area.

[a]Values are given as the mean ± standard deviation. Dissolved phosphorus and chlorophyll *a* concentrations are summer averages from June, July, and August samples. Standard deviations for invertebrate abundance are based on a sample size of five from samples collected in late August.

from July to early September. No fish were observed in these lakes during this period. Data on physical lake attributes and water chemistry (Table 1) were collected, analyzed, and reported by Jackson (2003).

The four lakes selected for this study differed systematically based on biological attributes measured over the summer (e.g., presence or absence of fish; Table 2). Lakes with fish appeared to be slightly more turbid and held lower concentrations of total phosphorus than lakes without fish (Table 1). All lakes were alkaline, with similar light penetration and dissolved organic carbon concentrations, and only SPA had a maximum depth greater than 2 m. SPA was smaller than the other three lakes, and conductivity in Dawson Lake was slightly higher than in other lakes.

Sample collection

All lakes were sampled in late August, when plants and their associated invertebrates were at or near their maximum seasonal biomass (Carpenter and Lodge 1986; Kornijów 1989). Macrophytes and epiphytic invertebrates were collected by a diver using SCUBA and a sampling device modified from Wollheim and Lovvorn (1995, 1996). The device was a plastic cylinder approximately 0.5 m tall, designed to capture aquatic macrophytes and associated invertebrates occurring within an area of 638 cm^2. The open end of the cylinder was gently lowered over the macrophytes being sampled. The opposite end was attached to a tapered canvas bag that led to a removable collection cup with a mesh size of 253 µm. Flouts were attached to the cup end to ensure that the sampler remained vertical in the water column during sampling. Three legs extended from the bottom of the cylinder to the lake bottom to allow a space of 10–15 cm above bare lake sediments. The open end of the sampler was equipped with a removable lid covered with 253-µm mesh to aid movement through the water column when the lid was attached. The lid was removed just above the top of the plants to minimize capture of organisms not associated with the macrophytes, then lowered carefully over plants until the sampler legs contacted the sediments. Plants were gently severed just above the sediments and the lid of the sampler was replaced. Care was taken to exclude sediments and associated benthic invertebrates from samples. The sampler was then inverted and brought to the surface, where water was drained through the collection cup. The plant material was removed by hand and placed in a sealable freezer bag. The sides of the sampler were rinsed with deionized water into the collection cup and the contents backwashed into the freezer bag containing the plants. A small amount of water was added to the sample, which was then placed on ice in a dark cooler for transport to the laboratory. Samples were stored in a refrigerator at 4 °C for up to 48 h before processing.

Distinct weed beds to be sampled in our lakes were identified as clear, continuous masses of aquatic macrophytes a minimum of 3 m across at the narrowest point, surrounded entirely by either open water or a combination of open water and shoreline. As all our lakes were relatively shallow (Table 1), distinct weed-bed patches were distributed throughout the entire lake and not limited to areas bordering the shoreline. This allowed the selection of independent weed beds for sampling from within our lakes.

We sampled three distinct littoral microhabitats — individual aquatic plants, weed-bed edges, and weed-bed centres — chosen to reflect the complexity of the surrounding environment in relation to the plants being sampled. An individual plant was defined and selected as a single macrophyte standing on bare sediment at least 2 m away from distinct weed beds. Weed-bed edges consisted of plants sampled on the edges of distinct weed beds. Weed-bed centres were defined as plants sampled from the middle of a dense weed bed, at least 1 m in from all edges to minimize edge effects. Five samples were taken in each habitat across the entire lake between the hours of 1000 and 1400. Weed beds were sampled only once, so that disturbance from taking a centre sample would not influence edge samples taken from the same weed bed, or vice versa. The positions of edge samples from around distinct weed beds were chosen randomly by an observer from a boat.

In the laboratory, samples were poured into a basin with a small amount of tap water. Plants were shaken free of invertebrates, spun in a salad spinner to remove excess moisture, and frozen for later analysis. The remaining debris and invertebrates were concentrated on a 253 µm mesh screen, transferred to containers, and preserved in 70% ethanol. Invertebrates were later sorted from debris and identified to order, suborder, family, or genus (following Clifford (1991)

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and Merritt and Cummins (1996)), enumerated, and then categorized according to functional feeding groups (following Merritt and Cummins (1996)).

Plants were thawed, separated, and identified to species following Fassett (1957), Burland (1994), and Moss (1983). Species names were then cross-referenced against the Integrated Taxonomic Information System (ITIS) online database (http://www.cbif.gc.ca/pls/itisca/taxaget?p_ifx=plglt) to report accepted scientific nomenclature. In cases where the ITIS nomenclature was different, we listed the more commonly employed scientific names in brackets behind the currently accepted ITIS conventions, as these may be more familiar to many of our readers. Any remaining invertebrates found on the macrophytes were categorized and enumerated. Plants were spun once more in a salad spinner to remove excess moisture and wet mass was measured.

Surface areas were estimated for plants from SPA and Dawson Lake. Representative plant shoots, stems, and leaves were measured to the nearest 1 mm on a minimum of 20 individual plants for each species. A digital image analyzer was used to measure leaf surface areas of broad-leaved species such as *Potamogeton richardsonii* (*Potamogeton richardsonni*). Averages for stem area and leaf area were calculated for each plant species. Stems and leaves were enumerated and surface area was calculated for the plants in each sample.

Defining measures of littoral habitat complexity

Aquatic macrophytes sampled typically reached the water surface, where they occurred in all our lakes. All samples were taken at depths of approximately 1.3 m (±0.2 m). Care was taken to ensure consistency of depth across samples taken within a lake, which was made easy by the highly regular bathymetry within our lakes: all sloped gently from their maximum depth to shoreline in a parabolic manner. Therefore, we assumed that the volume of the water column sampled was constant across all sampling sites in our lakes.

Plant surface area is a direct measure of the available substrate that littoral invertebrates can occupy within the volume of water sampled. Plant surface area was found to be strongly correlated with plant wet biomass in our study (Fig. 1*a*; $r = 0.957$, $p < 0.001$, df = 28). Based on the consistency of this relationship over two lakes and multiple plant species, and the relative ease with which plant wet biomass is measured relative to plant surface area, we chose plant wet biomass per square metre of lake bottom as our quantitative measure of habitat complexity. Thus, we used two independent measures of plant-habitat complexity in this study: microhabitat complexity and plant wet biomass per square metre of lake bottom. The first is defined categorically to describe the complexity of the plants sampled relative to their surrounding environment (i.e., individual plants versus weed-bed edges versus weed-bed centres). The second is defined continuously as wet plant biomass per square metre of lake bottom, which closely reflects plant surface area available to littoral invertebrates.

Statistical analyses

Total invertebrate abundance was $\log_{10}$-transformed to normalize distributions and homogenize variance among factors. The effects of fish (presence or absence; fixed effect), lake (SPA, SGC, Mushroom Lake, or Dawson Lake; random effect), and microhabitat complexity (individual plants, weed-bed edges, or weed-bed centres; fixed effect) on the total numbers of invertebrates encountered per square metre of lake bottom were tested categorically with a nested analysis of variance (ANOVA). Lake was nested within fish status, and we considered all higher order interaction terms. Sheffé's tests were used to evaluate significant terms among fixed effects in the ANOVA. We also used the same nested ANOVA model to test patterns of plant biomass across our qualitative determinations of habitat complexity. Plant biomass was fourth-root-transformed to satisfy assumptions of normality and homogeneity of variance among factors.

We also regressed invertebrate abundance against wet plant biomass for all lakes to examine patterns in total inver-

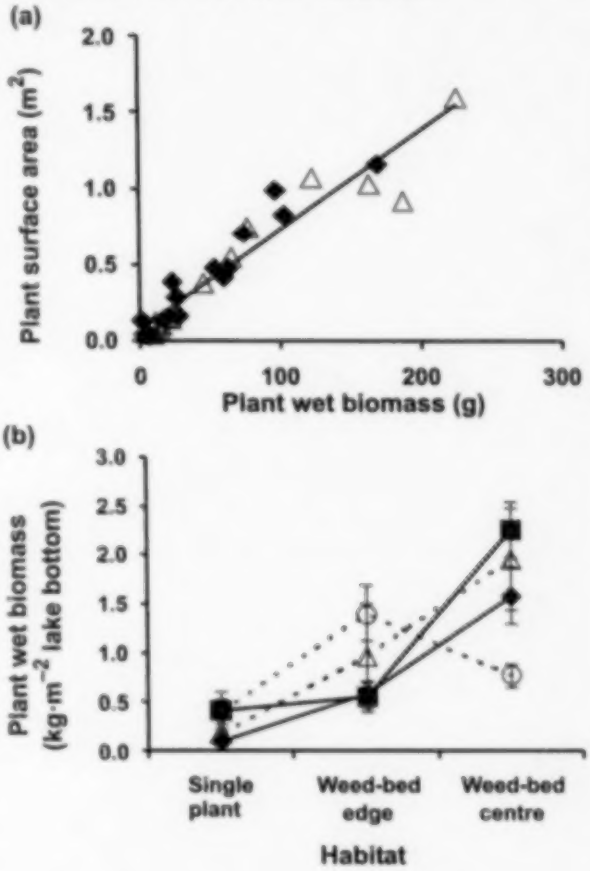

Fig. 1. Measures of aquatic macrophyte complexity in shallow prairie lakes (Strathmore Golf Course (■); Strathmore Picnic Area (◆); Mushroom Lake (○); Dawson Lake (△)). Open symbols represent fishless lakes and solid symbols indicate lakes with fish. (*a*) Correlation between macrophyte surface area (m^2) and macrophyte wet biomass (g) over 30 samples from two lakes. (*b*) Increase in plant biomass ($kg \cdot m^{-2}$ lake bottom) with habitat complexity (qualitative classification) for all lakes but Mushroom Lake. Error bars are ±1 standard error.

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Table 3. Taxonomic relationships among identified organisms that contributed more than 1% (on average) to their respective functional feeding group (FFG).

Taxonomy of identified specimens						
Phylum and subphylum	Class and subclass	Order and suborder	Family	Tribe and genus	FFG	Average frequency of occurrence in FFG (%)
Arthropoda						
Crustacea	Branchiopoda	Cladocera	Bosminidae		Filterers	9.12[S]
			Daphnidae		Filterers	12.3[T,S]
			Chydoridae		Filterers	18.2[S]
	Maxillopoda	Ostracoda			Detritivores	62.4[S*]
	Copepoda				Filterers	59.7[T,S*]
	Malacostraca	Amphipoda		*Hyalella* sp., *Gammarus* sp.	Detritivores	23.6[T]
Uniramia	Insecta	Diptera	Chironomidae	Non-Tanypodinae[b]	Scrapers	58.0[T,S*]
				Tanypodinae[b]	Predators	7.2[T,S]
			Ceratopogonidae	*Bezzia* sp.	Predators	3.4
		Ephemeroptera	Baetidae	*Baetis* sp., *Caenis* sp.	Scrapers	2.7[T]
		Odonata				
		Zygoptera			Predators	32.1[T*]
		Hemiptera	Corixidae	*Dasycorixa* sp.	Predators	1.8
Chelicertiformes	Chelicerata					
	Arachnida	Acari	Acara		Predators	15.5[T,S]
Annelida	Oligochaeta	Lumbriculidae			Detritivores	11.8[S]
	Hirudinea				Predators	3.0
Cnidaria	Hydrozoa	Hydroidea				
		Anthomedusae		*Hydra* sp.	Predators	17.3
Mollusca					Scrapers	31.7

Note: A superscript "T" denotes an item from the trout (*Oncorhynchus mykiss*) diet (Hodgson et al. 1991; MacNeil et al. 1999), and a superscript "S" denotes an item from the brook stickleback (*Culaea inconstans*) diet (Tompkins and Gee 1983) as determined by previous diet studies.

[a]The dominant taxa for each FFG.

[b]Chironomids were sorted only to this level of taxonomic separation. Though there is a diversity of FFGs at the genus and species levels within both groups of chironomids defined here, the FFGs assigned to these taxonomic divisions indicate the majority representation of species within each group (Paterson 1993; Merritt and Cummins 1996).

tebrate abundance against a continuous measure of habitat complexity. We compared the slopes and intercepts of the regressions between lakes with and without fish, and between lakes within a treatment. We corrected critical α values of our tests for the number of comparisons made. Effect sizes (defined here as the slope of $\log_{10}$ invertebrate abundance with plant biomass) were also compared between lakes to determine the maximum effect size of plant biomass on invertebrate abundance from fishless lakes in our study (Hoenig and Heisey 2001; Colegrave and Ruxton 2003; Di Stefano 2004).

To test the effects of lake and microhabitat on invertebrate community composition, invertebrates were sorted into zooplankton and other algal filter-feeders (hereinafter referred to as filterers), scrapers (feed on epiphyton), shredders (live on plant material), detritivores, and predators (Table 3). Functional feeding groups were represented as numerical proportions of the total invertebrate community to control for variation in total invertebrate abundance between samples. Proportional data often violate the assumption that groups are independent, and distributions of proportional data are frequently not normally distributed and therefore not appropriate for analysis by traditional parametric statistics (Jackson 1997). Arcsine square-root transformations normalized our proportional data. For each functional feeding group, we used the same nested ANOVA approach described previously to investigate differences among lakes and habitats (shredders, consisting largely of *Halplius* spp. or trichopteran larvae, were extremely rare in our samples and were thus excluded from this particular analysis).

Despite transformations, distributions of some functional feeding groups were found to be heteroscedastic. However, it has been argued that ANOVA is a robust statistical test and that even large departures from the parametric assumptions do not have a great impact on the results of the test, particularly when sample sizes are equal among groups (Zolman 1993; Zar 1999). Comparisons that were found to be heteroscedastic are indicated as such.

Results

Habitat complexity

Generally, plant biomass was best explained by microhabitat complexity (Fig. 1b, Table 4), although a high-order interaction term in the nested ANOVA model was significant (Table 4). With the exception of Mushroom Lake, all lakes demonstrated significant increases in plant biomass with increasing habitat complexity from individual plants to

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Table 4. Statistical results for individual factors in statistical ANOVA models examining patterns of invertebrate abundance and plant biomass across littoral habitats (single plants, weed-bed edges, weed-bed centres) and lakes, according to model 1.

Dependent variable	Statistical results for models	Habitat	Fish	Habitat × fish	Lake within fish	Habitat × lake within fish
Log_{10} invertebrate abundance	$F_{[11,48]} = 9.64$ $p < 0.0001^a$ $r^2 = 0.69$	0.022[a]	0.473	0.035[a]	0.312	0.086
Plant wet biomass	$F_{[11,48]} = 9.29$ $p < 0.0001^a$ $r^2 = 0.68$	0.020[a]	0.736	0.432	0.613	0.019[a]

[a]Significant at the 0.05 level.

weed-bed centres (Sheffé's test, between mean squared error (MSE) = 0.77697, df = 48; individual plants vs. weed-bed centres: Dawson Lake, $p = 0.021$; SPA, $p = 0.002$; SGC, $p = 0.038$; Mushroom Lake, $p > 0.05$).

Dawson Lake was dominated by *Ruppia maritima* and *Stuckinia pectinatus* (*Potamogeton pectinatus*); *Myriophyllum sibiricum* (*Myriophyllum excalbescens*), *S. pectinatus*, and *Potamogeton pusillus* dominated SGC and Mushroom lakes; and *S. pectinatus*, *P. richardsonii*, and *Myriophyllum sibiricum* were dominant in SPA. Plant condition, based on visual inspection, was similar in all samples. Systematic differences between plant species composition from one habitat to the next within lakes were not apparent. Owing to the number of samples examined from each habitat within a lake (5) relative to the number of plant species encountered (6), we were unable to assess statistically the effect of plant species composition on invertebrate abundance within habitats. It is not likely that changes in plant species composition and architecture across habitats contributed to our observations, since plant biomass typically increased by an order of magnitude between microhabitats in lakes (Fig. 1*b*). We assume that these large changes in plant biomass, and therefore surface area (Fig. 1), between microhabitats would have a much greater effect on the amount of substrate available to invertebrate assemblages than minute changes in plant architecture that may have occurred over this gradient because of differences in plant species assemblage.

Total invertebrate abundance

Invertebrate abundance per square metre of lake bottom increased significantly with increasing microhabitat complexity in lakes with fish only (Fig. 2*a*). A significant interaction found between fish presence and habitat type in the statistical model (Table 4) demanded further exploration with post-hoc comparisons. Invertebrate abundance did not differ significantly between habitat types in fishless lakes (a posteriori comparison of weed-bed centres = weed-bed edges = individual plants, Sheffé's test, MSE = 0.06516, df = 48, all $p > 0.05$). In contrast, invertebrate abundance increased significantly from individual plant habitats to weed-bed centres in lakes with fish ($p < 0.001$ for individual plants vs. weed-bed edges; $p = 0.009$ for weed-bed edges vs. weed-bed centres).

We used regression and analysis of covariance (ANCOVA) to explore relationships between our continuous measure of littoral habitat complexity (plant biomass) and invertebrate abundance (Fig. 2*b*), independent of our qualitative designations of microhabitat complexity. Lakes with fish demonstrated a significantly different slope from those without fish (test for heterogeneity of slopes, $F_{[1,56]} = 13.17$, $p = 0.0006$; $p_{crit} = 0.0073$ for seven comparisons). The

Fig. 2. Log_{10} invertebrate abundance (no.·m^{-2} lake bottom) plotted against habitat complexity in shallow prairie lakes (Strathmore Golf Course (■); Strathmore Picnic Area (◆); Mushroom Lake (○); Dawson Lake (△)). Habitat complexity is represented (*a*) categorically by sampling location or (*b*) continuously by wet plant biomass. Open symbols represent fishless lakes and solid symbols represent lakes with fish. Error bars are ±1 standard error.

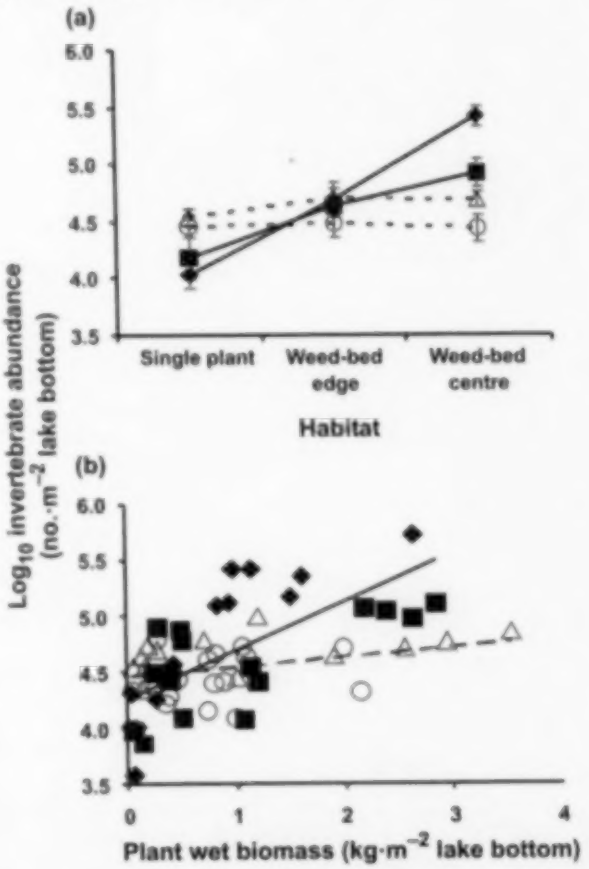

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Table 5. *P* values for factors in significant statistical models examining littoral invertebrate community patterns.

Dependent variable	Habitat	Fish	Habitat × fish	Lake within fish	Habitat × lake within fish
Filterer community	0.019[a]	0.884	0.272	<0.001[a]	0.149
Detritivore community	0.041[a]	0.444	0.196	<0.001[a,b]	0.580
Scraper community	0.127	0.892	0.218	0.022[a,b]	0.103
Predator community	0.307	0.143	0.172	0.037[a,b]	0.522

[a]Significance at 0.05.
[b]Heteroscedastic variance among factors.

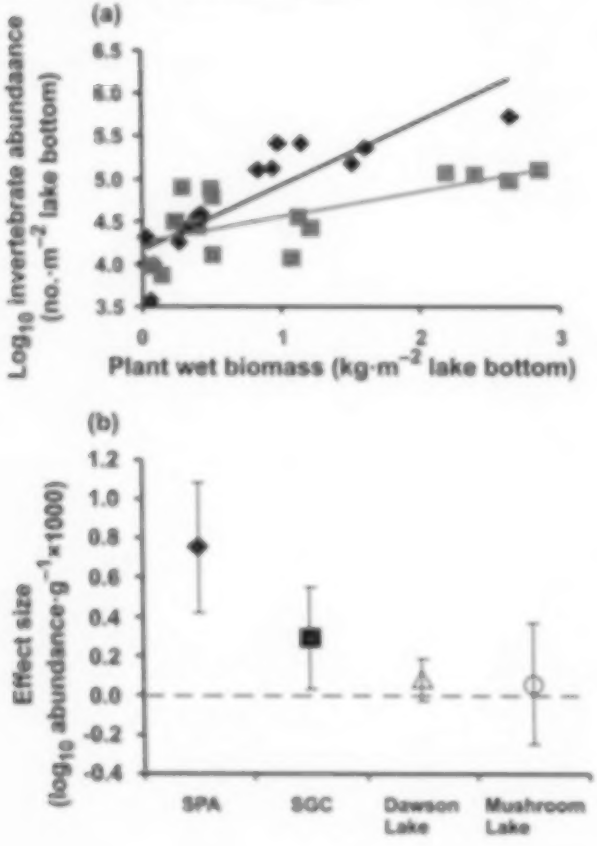

Fig. 3. (*a*) Log_{10} invertebrate abundance (no.·m^{-2} lake bottom) plotted against habitat complexity in lakes with fish only (Strathmore Picnic Area (SPA; solid symbols and line); Strathmore Golf Course (SGC; shaded symbols and line)). (*b*) Slopes ± 98.75% confidence intervals for effect size of plant biomass on log_{10} invertebrate abundance.

slopes of invertebrate abundance on plant biomass in lakes without fish were not significantly different from zero, nor were the intercepts different from one another (test: slope = 0; $p = 0.8700$ for Mushroom Lake, $p = 0.2000$ for Dawson Lake; test for equal intercepts, $p = 0.1062$; $p_{crit} = 0.0073$). In contrast, lakes with fish exhibited slopes significantly different from zero ($p = 0.0001$ for SPA, $p = 0.003$ for SGC; $p_{crit} = 0.0073$) and also differed from one another (test for heterogeneity of slopes, $F_{[1,52]} = 14.51$, $p = 0.0004$; Fig. 3*a*; $p_{crit} = 0.0073$).

To determine the maximum effect size of plant biomass on invertebrate abundance in fishless lakes, we examined confidence intervals around the slope of log_{10} total invertebrate abundance with plant biomass (Fig. 3*b*). We used 98.75% confidence intervals to correct for the number of comparisons made ($\alpha = 0.05/4 = 0.0125$; $\alpha_{one\text{-}tailed} = 0.00625$; confidence interval = $100 \times (1 - 2\alpha_{one\text{-}tailed}) = 98.75\%$) as recommended by Hoenig and Heisey (2001). Based on the largest confidence interval for fishless lakes (Mushroom Lake; Fig. 3*b*), we would not expect an effect of plant biomass on invertebrate abundance greater than 0.000364 log_{10} invertebrates/g wet plant biomass. This value overlaps considerably the 95% confidence intervals for both Dawson Lake and SGC, but is smaller than the lower confidence interval for SPA.

Invertebrate functional feeding groups

The proportion of invertebrate predators present in samples was best explained by variation in habitat and lake-specific effects (Table 5). Across all lakes, filterer communities were negatively influenced by increased habitat complexity (Table 5, Fig. 4*a*; Sheffé's test, MSE = 0.017, df = 48; individual plants < weed-bed edges = weed-bed centres). In lakes with fish, both detritivores and scrapers appeared to increase as a proportion of the invertebrate community as habitat complexity increased (Fig. 4). In fishless lakes, predators appear to make a more significant contribution to invertebrate communities in more complex habitats (Fig. 4*d*), even though total invertebrate abundance in fishless lakes did not differ between habitats (Fig. 2). This observation is of ecological interest because of the consistency of this pattern in both lakes without fish, and the clear absence of this pattern in lakes with fish (Fig. 4*d*).

To better understand the significance of this observation, and therefore the role of predators in the invertebrate community of dense weed beds, we performed a randomization test on the relative abundance of predators from weed-bed centres only to examine differences in this particular habitat among lakes. A randomization test was chosen for this analysis because it tests results against the actual distribution from which the data are sampled, and is therefore not sensitive to violations of normality or homoscedasticity. We compared the absolute value of the observed *t* statistic from the comparison of lakes with and without fish ($t = -3.67$) with 10 000 simulated *t* statistics generated from randomly sampling the observed values of transformed relative predator abundance. To account for the fact that these data were a subset of a larger set of data, we corrected our randomiza-

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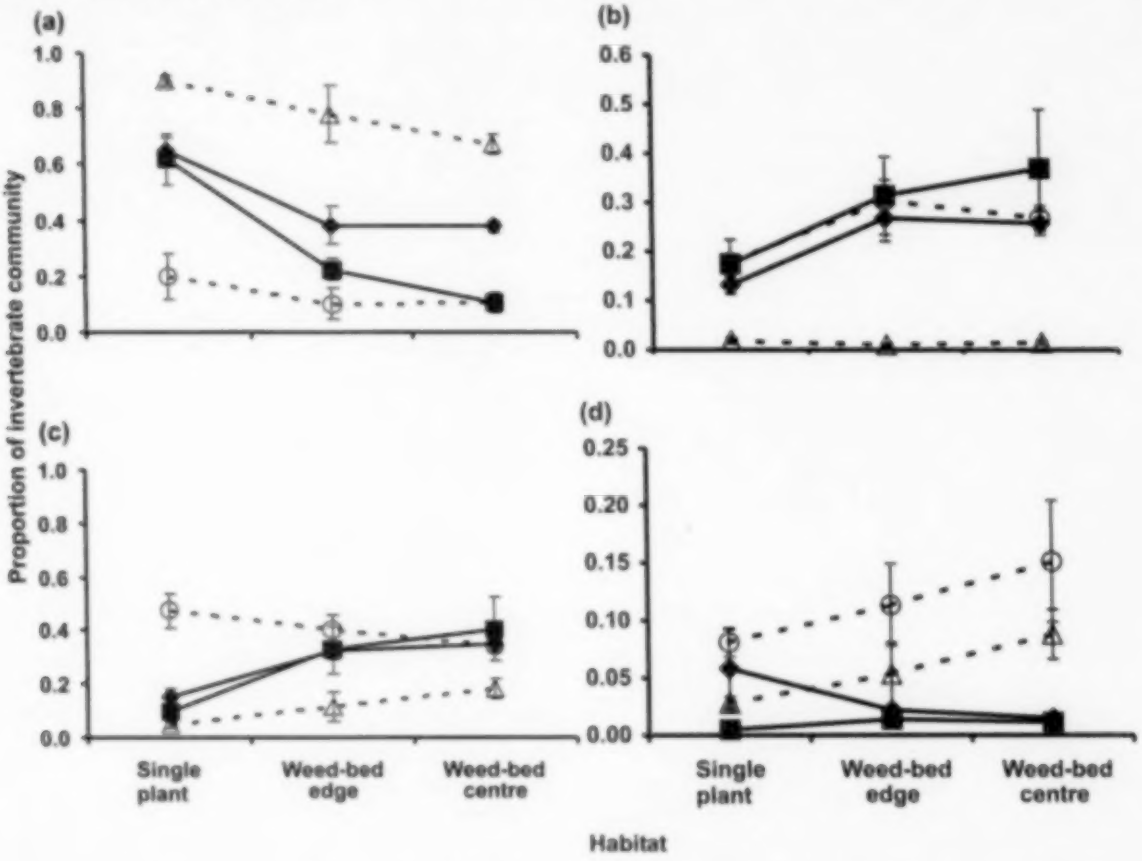
Fig. 4. Invertebrate community composition in shallow prairie lakes with and without fish (Strathmore Golf Course (■); Strathmore Picnic Area (◆); Mushroom Lake (○); Dawson Lake (△)). Open symbols represent fishless lakes and solid symbols represent lakes with fish. Patterns for (*a*) filterers, (*b*) detritivores, (*c*) scrapers, and (*d*) predators are shown. Error bars are ±1 standard error. Note the differences in scale on the *y* axis in *b* and *d*.



tion critical probability to $p_{crit} = 0.05/12 = 0.004$. The randomized p value from this procedure was 0; of 10 000 simulations, no single t value exceeded that of the observed test (the maximum t value obtained over 10 000 simulations was 3.18).

Discussion

Invertebrate abundance in fishless lakes was unrelated to either macrophyte microhabitat complexity or plant biomass. In contrast, over a similar range of plant species, plant biomass, and microhabitats, abundance of littoral invertebrates in lakes with fish was positively and strongly associated with gradients of habitat complexity. Positive relationships between invertebrate abundance and large-scale habitat complexity in lakes with fish are well documented in the literature (Cyr and Downing 1988*a*; Zimmer et al. 2000; Tolonen et al. 2001). Our data supported these previous findings and revealed that these patterns occur at much finer spatial scales than was previously considered. Addressing patterns of invertebrate distribution at this scale of investigation may be important when considering scales relevant to organisms occupying the littoral zone. For instance, some species of fish have been shown to prefer weed-bed edges to other littoral habitats when foraging for littoral invertebrates and (or) forage fish (Trebitz et al. 1997). It has also been suggested that fish foraging is maximized in weed beds of intermediate macrophyte density (Crowder and Cooper 1982). Experimental data have shown that high densities of artificial macrophytes significantly impeded fish foraging (Manatunge et al. 2000). Thus, the ability to accurately describe variation in invertebrate abundance at scales we consider here may have important consequences for modeling littoral invertebrates as prey for fish occupying the littoral zone.

Our study examines how invertebrates distribute themselves in weed beds when fish are absent — a condition that previous studies have not considered. Our findings suggest that trends in littoral invertebrate abundance in fishless lakes differ dramatically from the well-established positive relationship between total invertebrate density and habitat complexity in lakes with fish. The patterns we observed were consistent across both the fishless lakes we considered, and are supported by the limited experimental work that has

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been conducted on the topic. For example, Uiblein et al. (1996) reported that ostracods spent a significantly greater amount of time visiting aquatic macrophytes and less time in open water in the presence of a chemical fish cue than when it was absent. Thus, littoral invertebrates may be more closely associated with aquatic macrophytes in the presence of fish, but disperse more widely when this particular predation threat is absent. In contrast to the results of our study, Crowder and Cooper (1982) reported an increase in invertebrate density with increasing weed-bed density in fishless littoral enclosures. However, their results may reflect the movement of invertebrates across their fish-exclusion curtain (4.8 mm mesh) from areas with fish into fishless habitats.

A number of considerations suggest that the potential effect of macrophyte habitat complexity on invertebrate abundance in fishless lakes in this study is ecologically insignificant compared with effects observed in lakes with fish. First, our "fish" effect is likely conservative because both our lakes with fish had higher turbidity than those lacking fish. Rainbow trout and stickleback both rely on visual cues to detect prey (Croy and Hughes 1991; Flamarique and Browman 2001). However, increased turbidity can impair the predatory efficiency of fish (Utne-Palm 2002; De Robertis et al. 2003) and reduce their ability to detect littoral invertebrates in less complex habitats. We detected significant effects of fish presence despite this possibility.

Second, we detected a significant "fish" effect in two lakes with very different fish communities. The dietary preferences of stickleback and rainbow trout overlap considerably, though species-specific differences in body size are likely to generate differences in size-selective preferences for invertebrate prey. The relationship between invertebrate abundance and plant biomass had a shallower slope in SGC than in SPA, possibly reflecting the difference in fish community structure between lakes. Nonetheless, both relationships with habitat complexity were positive, and significantly different from those in fishless lakes. Additionally, the largest confidence interval for our fishless lakes suggests that the largest effect size supported by our data is 0.000364, which is lower than the lower bound of the confidence interval for SPA. Confidence intervals overlapped between SGC and our fishless lakes, which further supports the idea that the strength of fish effects on littoral invertebrate distributions likely depends on fish community structure.

Last, the narrow confidence intervals around the effect size (slope estimate) of plant biomass on invertebrate abundance in Dawson Lake, which includes zero, provides our best evidence that the null hypothesis of no effect between plant biomass and invertebrate abundance in fishless lakes is true. In other words, the inclusion of zero in the error around the slope estimate is not due simply to high variation in invertebrate density between sites sampled in this lake. Despite a consistent increase in plant biomass with habitat complexity in Dawson Lake, mean invertebrate densities were very similar among habitats. This suggests that our inability to detect an effect of habitat complexity on invertebrate abundance in Dawson Lake is less likely to be due to random variation masking a true effect. Our study suggests that the capacity for habitat complexity to affect invertebrate abundance in fishless lakes is too weak to be observable in fishless lakes at the scale of investigation in this study. However, it remains possible that habitat complexity may structure invertebrate communities in fishless lakes at spatial scales even finer than those investigated here.

In the most complex habitats, predators made up a greater proportion of the littoral invertebrate community in fishless lakes than in lakes with fish. Fishless lakes appeared to support a greater proportion of predators in more complex macrophyte habitats than in less dense habitats. This trend was significant, as evidenced by our randomization test. In lakes with fish, predators appeared to represent an equal proportion of the community across all habitats (SGC), or actually appeared to decrease as a proportion of the community as habitat complexity increased (SPA).

Significant differences in the contribution of predators to invertebrate communities between lakes with and without fish may be due to differences in invertebrate behaviour resulting from differential predation by fish. Like ostracods, some other invertebrates display alternative behaviour depending on whether fish are present or absent. Damselflies, the most common predatory invertebrate in our samples, display a "sit-and-wait" foraging behaviour in the presence of fish (McPeek 1990; Johnson 1991; Wellborn et al. 1996). This behaviour is adaptive for catching prey and avoiding predators. In environments that lack fish, damselflies are more active predators (Johnson 1991). Blois-Heulin et al. (1990) reported similar shifts between "cryptic appearance" and more active behaviour in damselflies from environments with and without fish, respectively. Damselflies are awkward swimmers (M. Rennie, personal observation) and in fishless lakes may find the pursuit of prey more effective across dense weed bed habitats.

The uniformity of invertebrate abundance among habitats in fishless lakes may be due to predation effects of invertebrate predators on nonpredatory invertebrates. Among the growing evidence of predatory effects of littoral invertebrate predators includes a report by Burks et al. (2001) that odonate nymphs were capable of reducing pelagic prey abundance (*Daphnia* sp.) in laboratory and field environments. Odonate nymphs and *Hydra* sp. have also been reported to significantly reduce rotifer abundance in laboratory experiments (Walsh 1995). In a fish removal experiment, Prejs et al. (1997) found depressed numbers of nonpredatory epiphytic invertebrates and more than a doubling of predatory invertebrates 2 years after fish removal. These studies support the hypothesis that in the absence of fish, invertebrate predators are capable of exerting strong negative predatory effects on nonpredatory littoral invertebrate communities. If this is so, predation — whether by fish or invertebrates — may be an important top-down mechanism in shaping the littoral invertebrate communities of shallow prairie lakes. This is particularly true for numerous shallow prairie lakes that harbour dense stands of macrophytes and are subject to high rates of oxygen depletion under ice and winter fish kills (Meding and Jackson 2001, 2003; Jackson 2003).

Proportions of filterers (primarily zooplankton) decreased as microhabitat complexity increased in all lakes. Other studies have shown similar negative relationships between zooplankton and aquatic macrophytes (Cyr and Downing 1988*a*; but see also Lauridsen and Buenk 1996). Given that

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samples were taken from each lake at similar times of the day, horizontal migration patterns of zooplankton would not result in any bias that could generate these results. This negative relationship between the relative abundance of filterers and habitat complexity might be a response to a reduction in suspended algal concentrations in macrophyte beds, brought about by shading from macrophytes (Carpenter and Lodge 1986). Aquatic macrophytes may also reduce water velocity (wind-generated currents in this case), causing algae to settle out into the sediment rather than remain suspended in the water column (Carpenter and Lodge 1986; Hamilton et al. 1990).

Distribution patterns of scrapers and detritivores in lakes with fish increased with littoral habitat complexity. No consistent trend for these two groups was apparent in fishless lakes. The pattern observed for scrapers and detritivores in lakes with fish may be a function of both top-down structuring via predation by fish and bottom-up energetic constraints. A number of the major taxa of scrapers and detritivores found in our samples are typical prey of both brook stickleback and rainbow trout. Mancinelli et al. (2002) found that the exclusion of large predatory fish resulted in an increase in the biomass and abundance of detritivores in experimental leaf packs (interestingly, those authors defined detritivores to include the scraper guild as well, though scrapers, considered on their own, showed no significant treatment effect in their study). However, scrapers feed primarily on epiphytic algae, which require a substrate to grow on (i.e., plants). As a result, scrapers may prefer dense macrophyte habitats with a large plant surface area per square metre of lake bottom. Likewise, detritivores are likely to find greater concentrations of decaying matter in dense weed beds than in open water because of deposition due to macrophyte senescence and the physical properties of weed beds on suspended particulate matter discussed above (Carpenter and Lodge 1986; Hamilton et al. 1990).

It is possible that other factors besides fish presence or absence generated the gradients of invertebrate distributions we measured. It could be argued that differences in water chemistry might generate the patterns we observed. For this to be true, however, chemical differences among lakes would need to also translate into persistent chemical gradients between habitats (i.e., water chemistry by habitat interactions). Additionally, these chemical gradients would have to occur in lakes with fish but not in lakes without fish (a three-way interaction between fish presence, water chemistry, and habitat). We suggest that this scenario is unlikely, since many shallow lakes, including those in our study, undergo frequent and thorough mixing (Blindow et al. 1993; Scheffer et al. 1993). Thus, horizontal chemical gradients between habitats are unlikely to persist long enough to form structural gradients in invertebrate distributions. Additionally, these lakes all occur within a few kilometres of one another and experience similar wind patterns and weather. Therefore, wind-generated mixing should also be similar between all lakes, and not differ between lakes with and without fish. The fact that our measured patterns in invertebrate distributions occur not just between lakes with and without fish, but across habitats within those lakes, suggests that there is a selectively structuring force (i.e., predators) in our study system.

In two recent mesocosm studies, water temperature, nutrient concentrations, and dissolved inorganic carbon have been shown to have positive effects on invertebrate abundance (Jones et al. 2002; McKee et al. 2003). However, both studies were only able to maintain these nutrient gradients by using mesocosm enclosures, which does not reflect the situation in shallow lake habitats in nature. Additionally, both studies demonstrated significant effects of fish (stickleback in one study, rainbow trout in the other) as large and as frequent as water-chemistry effects, and fish effects that were often more pronounced under high-nutrient and warm conditions (i.e., fish by warming or fish by nutrient interactions) — conditions that are consistent with those in the lakes in our study. Therefore, because shallow prairie lakes mix frequently, and all lakes in this study experience similar weather and climate, we believe that chemical gradients in these lakes are unlikely to persist over the time scales required to influence invertebrate abundance patterns directly, and that complex three-way interactions between water chemistry, habitat complexity, and fish presence are an unlikely and unparsimonious alternative explanation for our trends.

Though our study does not directly address how fish presence and habitat complexity interact to affect invertebrate biomass, our results, when considered alongside other findings, may offer some speculative insights. In lakes with fish, total invertebrate abundance increased by one order of magnitude or more with increasing habitat complexity. Other studies in lakes with fish have shown that more complex littoral habitats typically house larger bodied invertebrates than less complex habitats (Hanson 1990; Rasmussen 1993; Tolonen et al. 2003). If invertebrates in our lakes followed a similar pattern, it is very likely that total invertebrate biomass in lakes with fish follows a pattern similar to abundance, i.e., increasing with habitat complexity. In fishless lakes, though we did not observe differences in invertebrate abundance across gradients of habitat complexity, the proportion of predatory invertebrates increased by about 5% over the range of habitat complexity we measured. The dominant predatory invertebrates in this study were damselflies, and this may have resulted in increases in total invertebrate biomass with habitat complexity in fishless lakes; however, it is unclear whether a 5% increase in relative abundance, even in a relatively large-bodied predator, would be significant enough to cause a substantial increase in overall invertebrate biomass.

The functional assemblage of invertebrate communities changed with habitat complexity in each lake we studied. This implies that trophic linkages among invertebrates, and therefore ecosystem function, may also vary substantially across similar gradients of habitat complexity. Macrophyte beds are often assumed to be homogeneous with respect to the invertebrate communities they support (Cyr and Downing 1988*a*, 1988*b*; Zimmer et al. 2000). Yet our data suggest that variation in littoral invertebrate community and abundance patterns should be considered at smaller spatial scales — which are perhaps more relevant to the organisms ecologists wish to describe — to better understand patterns of energy flow through these heterogeneous systems. In particular, variability in predation pressure in the littoral zones

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of highly productive shallow lakes (via invertebrates or fish) and systematic differences in habitat complexity (and thus prey availability) appear to interact significantly. In doing so, these factors may regulate invertebrate abundance patterns in the littoral zones of lakes and may also play an important role in diversifying the ecological function of the invertebrate community across variably complex habitats.

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Empirical study of cyanobacterial toxicity along a trophic gradient of lakes

A. Giani, D.F. Bird, Y.T. Prairie, and J.F. Lawrence

Abstract: A series of 22 lakes in southern Quebec spanning a wide trophic range were sampled to develop models of changes in cyanobacterial abundance and toxicity. All lakes contained toxic cyanobacteria, and epilimnetic toxin content, expressed as microcystin equivalents, was best predicted by total nitrogen concentration and total phosphorus concentration (TP). Although phytoplankton biomass increased linearly with increases in TP among lakes, toxigenic biomass increased as greater than the squared power of TP. The only potentially toxigenic genera whose biomass was correlated with microcystin concentration were *Microcystis* and *Anabaena*. Surprisingly, the best model for toxic-species biomass was based on epilimnetic nitrogen. The level of the hepatotoxin microcystin per unit biomass in these organisms did not vary markedly among lakes, supporting the idea that environmental factors control the occurrence, but have only a limited effect on the toxicity, of potentially toxic species.

Résumé : Nous avons échantillonné une série de 22 lacs du sud du Québec couvrant une large gamme trophique afin de mettre au point des modèles du changement d'abondance et de toxicité des cyanobactéries. Tous les lacs contiennent des cyanobactéries toxiques et le contenu de toxines dans l'épilimnion, exprimé en équivalents de microcystine, est prédit le mieux par les concentrations d'azote total (TN) et de phosphore total (TP). Bien que la masse du phytoplancton s'accroisse de façon linéaire en fonction de l'accroissement de TP parmi les lacs, la biomasse des toxigènes augmente plus rapidement que la puissance au carré de TP. Les seuls genres potentiellement toxigènes dont la biomasse est en corrélation avec la concentration de microcystine sont *Microcystis* et *Anabaena*. De façon inattendue, le meilleur modèle de la biomasse des espèces toxiques est basé sur l'azote de l'épilimnion. La concentration chez ces organismes de l'hépatotoxine microcystine par unité de biomasse ne varie pas considérablement d'un lac à l'autre, ce qui appuie la proposition que les facteurs du milieu contrôlent la présence des espèces potentiellement toxiques, mais qu'ils ont un effet limité sur leur toxicité.

[Traduit par la Rédaction]

Introduction

Cyanobacteria are highly successful photoautotrophic prokaryotes of ancient lineage. They are a common feature of many aquatic systems including tropical and temperate water bodies. Cultural eutrophication of many water bodies has led to changes in the phytoplankton community with the development of greater biomass of algae, especially cyanobacterial populations. Mass developments, or blooms, of planktonic cyanobacteria frequently occur in fertile waters when warm stratified conditions permit (Paerl 1996). Many authors have concluded that the success of these organisms cannot be explained by a single characteristic but that several intrinsic and environmental factors work together to favour blooms (Paerl 1996; Hyenstrand et al. 1998; Oliver and Ganf 2000). Harmful algal blooms in a lake ecosystem are typically characterized by the heavy biomass accumulation of one or a few species. Roelke and Buyukates (2002) suggest that blooms represent a deviation from "normal" ecological systems, since they cause a disruption in the normal pattern of phytoplankton succession. In this situation diversity decreases, changing the interactions within the whole aquatic community from virus through zooplankton to fish (Romo and Miracle 1995; Zohary et al. 1996; Figueredo and Giani 2001). Cyanobacterial communities tend to create self-regulating populations (Hambright and Zohary 2000) characterized by a high degree of resilience that prevents ecosystem recovery to lower biomass levels.

Several species of cyanobacteria have the unfortunate ability to produce toxins, including hepatotoxins, neurotoxins, cytotoxins, and dermatotoxins. One major group of toxins is cyclic heptapeptides called microcystins, which are powerful hepatotoxins (Carmichael 1994). The general structure of microcystins is cyclo-(-D-Ala-*X*-D-MeAsp-*Z*-

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Adda-D-Glu-Mdha-), where *X* and *Z* are variable L-amino acids, D-MeAsp is D-*erythro*-β-methylaspartic acid, and Mdha is *N*-methyldehydroalanine. Adda is a 20-carbon nonprotein amino acid that is important for the toxicity of the compound. Microcystins inhibit protein phosphatase and are known to act as tumour promoters (Falconer and Humpage 1996). Acute animal and more rarely human poisonings have been reported worldwide, resulting from ingestion of water containing cyanobacterial cells (Lambert et al. 1994; Dow and Swoboda 2000).

The capacity of some cyanobacteria to produce toxins, and the presence/absence of toxicity in blooms, present both theoretical and practical challenges for aquatic scientists. What role do toxins perform for their producers? Are there special conditions that favour or control toxin production? It is possible that the toxicity of the community depends on the same environmental factors that favour cyanobacterial dominance (Jacoby et al. 2000; Kotak et al. 2000). Laboratory experiments performed on a variety of factors (Rapala et al. 1997; Lee et al. 2000; Oh et al. 2000) and several field studies (Vezie et al. 1998; Jacoby et al. 2000; Kotak et al. 2000) have focused on the effect of individual parameters on toxin production (for a review see Sivonen and Jones 1999). Production of cyanobacterial secondary metabolites, including toxins, may be regulated by environmental or endogenous factors affecting physiological state, growth stage, and biosynthesis (Paerl and Millie 1996). It is not entirely clear, however, whether the role of any or all toxins is primarily defensive; at least some seem to be constitutive and important for growth (Orr and Jones 1998; Lyck 2004).

In the present study, 22 water bodies in Quebec, Canada, of contrasting chemical and physical characteristics were surveyed to assess microcystin production and identify associated environmental factors. The variables examined in these systems included nutrient fractions (dissolved inorganic, dissolved organic, and total) for phosphorus and nitrogen, nutrient ratios, pH, temperature, dissolved free carbon dioxide, lake mean depth and water retention time, light-extinction coefficient, and the abundances of different cyanobacterial genera.

Material and methods

Sampling

Twenty-two lakes in the St. Lawrence lowlands, in the Eastern Townships (Appalachian geological province), on the Laurentian shield, and in Abitibi–Temiscamingue (western Quebec) were sampled between mid-June and mid-August 2001. A single integrated water sample was taken each time a lake was visited. Twenty-one of the lakes were sampled once and one was sampled (Lac St. Georges) twice. Lakes were chosen to represent a gradient of fertility, from oligotrophic (4.9 µg total phosphorus (TP)·L^{-1}) to eutrophic (130 µg TP·L^{-1}). Integrated water samples were collected at a central station by combining 1-m-long van Dorn bottle samples from all depths of the epilimnion in a clean polypropylene bottle, and subsampling after thorough mixing. Triplicate samples for measuring nutrients were taken from these samples. Samples for measuring dissolved nutrients were filtered immediately through 0.45-µm cellulose acetate filters and preserved with sulfuric acid for transport to the laboratory. TP and total dissolved phosphorus concentrations (0.2-mm-filtered water) were measured by the molybdenum-blue method after persulfate digestion. Total nitrogen (TN) and total dissolved nitrogen (0.2-mm-filtered water) concentrations were measured as nitrates after alkaline persulfate digestion. All colorimetric analyses were carried out on an Alpkem RFA300 or a Flow Solution IV autoanalyzer, for which detailed methodologies are given in Cattaneo and Prairie (1995). Concentrations of dissolved organic carbon in 0.2-mm-filtered sample water were measured by high-temperature oxidation on an OI Analytical TIC-TOC analyzer using persulfate oxidation after acidification. Temperature and oxygen profiles were obtained with a WTW Oxi 197 DO meter. Transparency (Z_{SD}) was measured with a 20-cm Secchi disk and the light-extinction coefficient (kd) was estimated using Poole and Atkins' (1929) formula: $kd = 1.7/Z_{SD}$. This transformation provided information on the amount of light received by the plankton, and linearized the relationships based on light.

Watershed and morphometric variables were determined using GIS analysis of government digital maps and our digitization of lake bathymetric maps (Prairie and Soucisse 1999). Water-renewal times were calculated using mean annual precipitation minus evaporation, based on Quebec Ministry of the Environment data.

Chlorophyll *a* concentration was determined by filtration onto 47-mm GFF filters, extraction of pigments by hot 90% ethanol, and measurement of fluorescence (Nusch 1980; Riaux-Gobin and Klein 1993). Since many lakes contained a substantial fraction of chlorophytes, we did not correct chlorophyll *a* values for phaeopigments because of potential interference from chlorophyll *b*, which leads to underestimation of chlorophyll *a* and overestimation of phaeopigments (Gibbs 1979).

Phytoplankton samples for enumeration were collected at the same time as nutrient samples, and 100 mL was preserved with 0.5 mL of Lugol's iodine. Counts were done under an inverted microscope by Utermöhl's method (Utermöhl 1958). Algal biovolume was calculated from single cells according to Rott (1981) and converted to carbon biomass with the equations of Menden-Deuer and Lessard (2000). Concentrated plankton samples for microcystin determination were collected by a 35-µm-mesh plankton net. Water samples for estimating dry weight were filtered onto preweighed 47-mm GF/F glass-fiber filters.

Determination of microcystin concentration

Microcystin concentration was determined on lyophilized samples. Extraction was performed with 75% *v/v* methanol (Fastner et al. 1998). A weighed sample of freeze-dried material was extracted in a 2-mL microcentrifuge tube by adding 1.5 mL of solvent, sonicating, shaking for 10 min, and centrifuging at 3500 rpm. The extraction procedure was repeated twice and extracts were combined.

Analyses of microcystin content were carried out by the protein phosphatase inhibition assay and by high-performance liquid chromatography (HPLC). The inhibition assay measures the microcystin-dependent inhibition of the phosphatase enzyme in vitro. In the colorimetric assay (An and Carmichael 1994; Rivasseau and Hennion 1999), protein phosphatase-2A (PP2A) activity against *para*-nitrophenyl

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phosphate (pNPP) is determined by quantitation of *para*-nitrophenol (pNP) hydrolyzed from pNPP. The pNP is estimated by absorbance at 405 nm using a microplate reader. The assay measures total PP2A inhibition without differentiating between different structural variants of microcystin and is calibrated with a microcystin-LR (MC-LR) standard curve, so microcystin content is expressed in MC-LR equivalents. Thus, in this paper microcystin concentrations represent the total toxicity of samples in MC-LR equivalents.

Phosphatase results were verified by HPLC analyses performed in the laboratories of Health Canada in Ottawa on a subset of 12 lakes. An immunoaffinity column was used for sample purification (Lawrence and Menard 2001). The use of antibodies for purification of the samples increased the sensitivity to the level of 1 ng·g^{-1}. After extraction and dehydration, microcystin samples were diluted with acetonitrile and run on a reverse-phase C18 silica column with separation achieved over a gradient of water and acetonitrile. Results obtained by these two methods were positively correlated ($r^2 = 0.72$) and there were no major discrepancies.

An attempt was made to account for the presence and quantity of toxin in lake samples by correlation with the biomass of those species or genera that had previously been shown to have toxin-producing strains. The toxigenic genera included in the analysis included species of *Microcystis*, *Anabaena*, *Radiocystis*, *Aphanocapsa*, and *Oscillatoria*/*Planktothrix*. Total toxigenic biomass was estimated as a toxicity-weighted function of the biomasses of certain genera, as described in the Results. The variability of microcystin content per unit toxigenic biomass was calculated by dividing microcystin content per litre by total toxigenic biomass per litre. In a few lakes, it was possible to measure microcystin, although no known toxic species or very low concentrations of them were recorded in the phytoplankton samples. Several explanations are possible: some species of cyanobacteria, at present not known to be toxigenic, may also produce microcystin; the enzyme phosphatase may have suffered some inhibition other than by microcystin; or, more likely, the net sample we used for toxin analyses may have collected some colonial species that were not observed in the phytoplankton samples used for counting and biomass calculation.

Statistical analysis

To investigate the relationship between microcystin concentration and the independent variables, we performed correlation and univariate and multiple regression analyses on appropriately transformed data. Significant relationships were defined as $p < 0.05$. Transformations were chosen by Box–Cox maximum-likelihood optimization (Box and Cox 1964) followed by residual analysis (Draper and Smith 1980) in order to meet normality and linearity assumptions. The result was that all variables were log-transformed except pH and temperature, which were unchanged, and the light-extinction coefficient, which was transformed to the square root. Multiple regressions were performed by backwards stepwise procedures starting with all variables entered, using probability to leave of 0.10, but all coefficients of final models were significant at the $\alpha = 0.05$ level at least. Analysis of covariance (ANCOVA) was used when the goal was to compare regression slopes among groups. When log–log regression slopes greater than 1 were compared with a null hypothesis constant of 1 to test for arithmetic linearity, a *t* test was used. When log–log slopes less than 1 were similarly tested for linearity, in order to be conservative, *t* tests were based on model II (reduced major axis) slopes to account for attenuation biases due to error in *X* (Laws and Archie 1981). Analyses were run with the statistical package JMP® for Windows, version 5.1 (SAS Institute Inc. 2003).

Results

The physical and chemical data gathered during this study are summarized in Table 1. The lakes presented a wide range of trophy, with TP varying from 4.9 µg·L^{-1} (Brompton) to 130.0 µg·L^{-1} (Abitibi). The highest chlorophyll *a* concentrations were found in Tomcod Lake (31.0 µg·L^{-1}) and the lowest in Truite Lake (1.4 µg·L^{-1}). The lakes ranged from shallow (Z_{max} = 1.5 m) to moderately deep (Z_{max} = 57.9 m). Estimated water-retention time was relatively short, 0.04–8.6 years.

Three variables showed interesting relationships with lake plankton toxicity — TP, TN, and phytoplankton taxonomy — and these variables form the basis of this paper. When multiple regression was used to partial out nutrient effects by including TN and TP, there were no significant relationships, independent of TN and TP, between microcystin content and pH ($p = 0.38$), free carbon dioxide ($p = 0.26$), dissolved nutrient fractions (total dissolved phosphorus concentration: $p = 0.23$; total dissolved nitrogen concentration: $p = 0.79$; dissolved organic nitrogen: $p = 0.60$; NH_4: $p = 0.41$; NO_3: $p = 0.35$), lake morphometry (residence time: $p = 0.94$; mean depth: $p = 0.85$), or light-extinction coefficient ($p = 0.38$). Although microcystin content per litre was strongly related to TN and TP individually and together, it showed no relationship to the TN/TP ratio ($r^2 = 0.02$, $n = 23$, $p = 0.52$).

The relationship between TP in the studied lakes and the biomass of total phytoplankton, total cyanobacteria, and potential toxigenic species is illustrated in Fig. 1. In this instance, potential toxigenic biomass represents the unweighted sum of the biomasses of species of *Microcystis*, *Anabaena*, *Oscillatoria*, and *Aphanocapsa*. Comparing the slopes of the log–log relationships indicates that, with increasing TP, changes are much faster for cyanobacterial biomass (nearly quadratic; slope 1.96) than for total phytoplankton (nearly linear; slope = 1.16) and fastest for the potential microcystin-producing component of the community (slope = 2.17). The two cyanobacterial components increased significantly faster than did biomass (ANCOVA: $t = 2.56$, $p = 0.01$) but did not differ from each other ($t = 0.84$, $p = 0.40$). This implies that small increases in phosphorus concentration in the water may produce rapid changes in the toxicity of the resulting algal assemblage. At the same time, there was no indication in these data that microcystin producers increase as a fraction of total cyanobacteria along the trophic gradient.

We compared our data on changes in microcystin concentration per unit volume relative to TP with the data of Kotak et al. (2000) from several lakes in western Canada (ANCOVA; Fig. 2). Our results show that a similar relationship holds, in both slope ($p = 0.73$) and elevation ($p = 0.30$), in all these lakes without regard to geography or trophic sta-

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Table 1. Physical and chemical characteristics of the studied lakes.

Lake	TP (μg·L^{-1})	TDP (μg·L^{-1})	TN (μg·L^{-1})	NO_3^- (μg·L^{-1})	NH_4^+ (μg·L^{-1})	DON (μg·L^{-1})	TN/TP (molar)	Chlorophyll *a* concentration (μg·L^{-1})	Z_{max} (m)	Z_{mix} (m)	Lake area (km^2)	Residence time (years)	MC-LR (μg·L^{-1})
À l'Ours	29.5	18.7	576	2.8	32.8	462	43.2	4.0	16.0	8.0	0.15	nd	0.009
Abitibi	130	nd	950	nd	nd	nd	16.2	60.0	15	nd	224	nd	0.41
Bowker	7.5	5.2	486	0	23.6	172	143.5	1.9	57.9	23.9	2.45	8.6	0.0017
Brompton	4.9	2.1	541	85.6	1.4	300	244.5	3.5	42.4	11.0	11.71	1.5	0.071
Brunet	41.5	23.9	588	2.8	100.0	331	31.4	9.8	1.5	1.0	0.24	0.32	0.013
Croche	6.9	5.2	755	8.4	8.7	298	242.3	1.5	11.0	5.3	0.18	nd	0.0031
Cromwell	9.8	7.5	588	2.5	9.4	458	132.9	6.3	7.5	2.0	0.09	nd	0.0008
Denison	47.1	20.5	691	0	40.9	417	32.5	21.3	5.0	1.6	0.3	0.04	0.054
Fraser	10.3	7.6	663	4.6	19.6	248	142.5	2.8	19.8	8.6	1.62	0.36	0.19
Hency	18.0	nd	410	nd	nd	nd	50.4	3.0	32		12.5	nd	0.0022
Libby	20.8	6.9	397	2.9	13.8	264	42.3	14.9	3.1	1.1	0.43	0.16	0.010
Lovering	16.5	10.9	437	33.3	19.5	332	58.6	3.9	25.0	9.8	4.9	1.6	0.0021
Montjoie	13.8	13.1	378	0	20.1	250	60.6	4.6	21.3	8.0	3.3	2.9	0.0022
Morency	24.2	12.2	356	3.2	30.0	321	32.6	2.4	20.0	7.5	0.26	1.4	0.0055
Parker	18.5	9.6	624	2.9	17.3	488	74.7	11.2	9.1	3.6	0.23	0.05	0.046
Simoneau	12.9	8.6	389	2.9	0	357	66.8	3.2	24.4	9.3	0.45	0.41	0.0010
St. Georges	46.1	14.0	901	3.6	21.5	469	43.3	41.8	4.3	1.8	0.51	0.18	0.23
Stukely	6.1	4.8	302	12.6	23.9	218	109.6	2.1	30.5	13.1	4.00	4.0	0.0032
Tomcod	58.5	39.6	834	2.8	37.5	592	31.6	31.0	1.8	0.9	0.81	0.06	1.91
Trois Lacs	36.9	16.0	681	34.6	30.5	458	40.9	19.3	10.1	1.3	2.4	0.01	0.076
Truite	31.7	8.5	449	3.5	50.9	256	31.4	1.4	12.2	3.3	2.4	0.30	0.046
Walfred	35.6	6.0	376	3.1	86.8	191	23.4	3.6	4.0	1.8	0.15	0.34	0.011

Note: Values for water-quality variables generally refer to July–August 2001 (nd, not determined). TP, total phosphorus concentration; TDP, total dissolved phosphorus concentration; TN, total nitrogen concentration; NO_3^-, nitrate; NH_4^+, ammonium; DON, dissolved organic nitrogen; TN:TP, ratio of total nitrogen to total phosphorus concentration; Z_{max}, lake maximum depth; Z_{mix}, epilimnion thickness; MC-LR, microcystin equivalents in the epilimnion.

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Fig. 1. Relationships between log total phosphorus concentration (TP) and log biomass of phytoplankton (—●—), total cyanobacteria (- - -○- - -), and potentially microcystin-producing cyanobacteria (—■—). The respective regression equations are as follows: log phytoplankton biomass = –5.4 + 1.16 log TP, r^2 = 0.60; log cyanobacterial biomass = –7.3 + 1.96 log TP, r^2 = 0.64; log potential toxigenic biomass = –8.1 + 2.17 log TP, r^2 = 0.69 (biomass units for the equations are grams of carbon per litre).

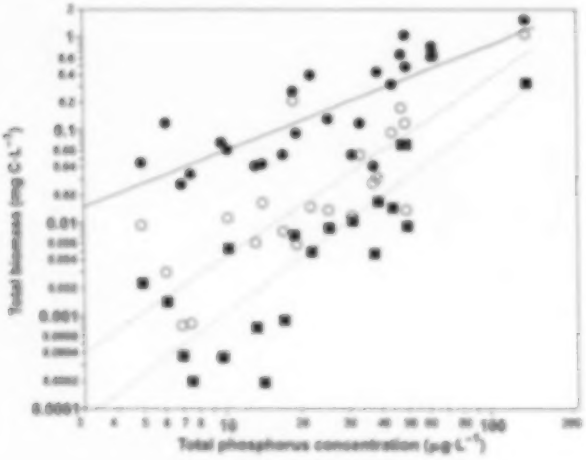
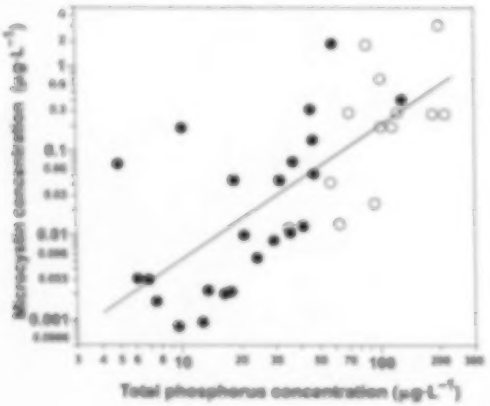


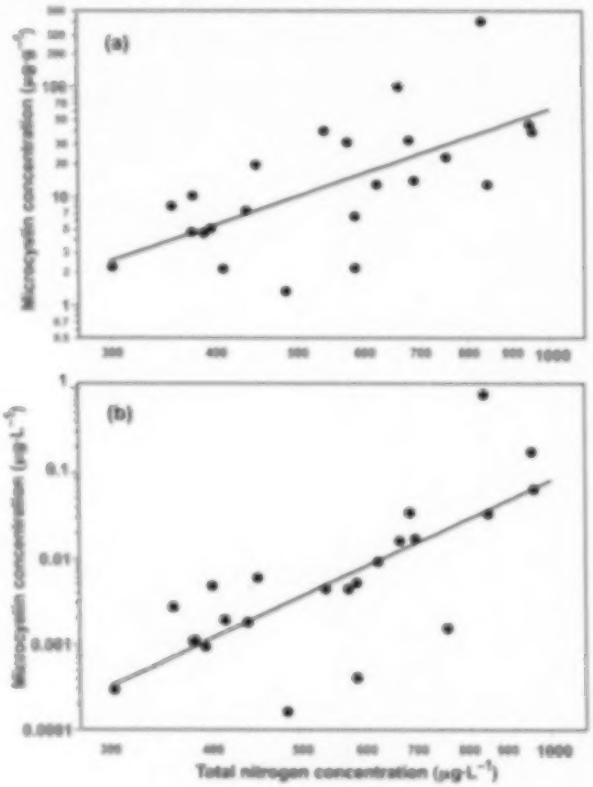
Fig. 2. Positive relationship between log total phosphorus concentration and log microcystin concentration in southern Quebec (this study; ●) and the summer mean for eutrophic lakes in Alberta (Kotak et al. 2000; ○). Lakes in both regions follow the same trend.



tus. The general equation was log(microcystin concentration ($\mu g \cdot L^{-1}$)) = –3.88 + 1.59 log TP ($\mu g \cdot L^{-1}$), $r^2 = 0.53$, $n = 36$, $p < 0.0001$. The slope of this log–log relationship was significantly greater than linear (*t* test, $p < 0.02$), indicating the increasing relative importance of toxic species in richer lakes.

In our lakes, TN also showed positive significant relationships with microcystin concentration expressed in either micrograms per gram (Fig. 3*a*) or micrograms per litre (Fig. 3*b*). Increasing TN favours, or results from, a greater

Fig. 3. Scatter plots and regressions between log total nitrogen concentration (TN) and (*a*) sestonic log microcystin concentration and (*b*) epilimnetic log microcystin concentration in lakes of southern Quebec. Regression equations are log microcystin concentration ($\mu g \cdot g^{-1}$) = –6.2 + 2.66 log TN, $r^2 = 0.43$, $p = 0.0007$ for *a* and log microcystin concentration ($\mu g \cdot L^{-1}$) = –14.8 + 4.57 log TN, $r^2 = 0.56$, $p < 0.0001$ for *b*.



fraction of toxigenic species in the plankton, which increases the toxicity of the seston (Fig. 3*a*). Similarly, greater toxicity per unit volume of the water column is associated with more nitrogen (Fig. 3*b*).

To distinguish the relative importance of phosphorus and nitrogen for microcystin production, and to compare these results with trophic effects of an increase in phosphorus on the biomass of the phytoplankton, multiple regressions were calculated and *F* values compared. As expected, lake chlorophyll *a* content was best predicted by TP ($p = 0.003$) and secondarily by TN ($p = 0.01$). But unexpectedly, when predicting lake microcystin concentration, TP was relatively weakly significant in the multiple regression ($p = 0.02$), whereas TN was highly significant ($p = 0.001$). The equation was

$$\log(\text{microcystin concentration}) = -12.6 + 0.9 \log \text{TP} + 3.5 \log \text{TN}$$

where all variables are expressed in micrograms per litre ($R^2 = 0.59$, $n = 23$, $p < 0.0001$). Despite the moderately strong relationships between the absolute concentrations of

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both nitrogen and phosphorus and microcystin concentration, no significant relationship was found here between microcystin concentration and the nitrogen:phosphorus ratio, as was noted above. This result is contrary to what has been seen periodically elsewhere (see Discussion). We should point out here that the range of TN/TP ratios in these Quebec lakes, starting at the Redfield molar ratio of 16 and going to 245 (Table 1), is within the range thought to indicate phosphorus, but not nitrogen, limitation.

The microcystin content of these lakes could be predicted on the basis of the biomass (g C·L^{-1}) of only two genera, *Microcystis* and *Anabaena*. The regression equation

$$\text{microcystin concentration } (\mu g \cdot L^{-1}) = 3560(\textit{Microcystis}) + 2600(\textit{Anabaena})$$

explained 95% of the variation in toxin content ($F = 28.8$, $p < 0.0001$). *Microcystis* biomass had the greatest effect in the equation ($t = 13.15$ versus $t = 8.52$ for *Anabaena*). Using the molecular weight of microcystin (995.17) and its carbon content (49 atoms of carbon) we can estimate the average toxin fraction of *Microcystis* as 0.21% of its cell carbon. *Anabaena*'s toxin content was 0.15% of its cell carbon. Therefore, on average, *Anabaena* species were 70% as toxic as *Microcystis* species. There were eight lakes with apparent microcystin content but no evidence of *Microcystis* or *Anabaena*. These lakes had a low toxin content and contributed only to the error in the equation.

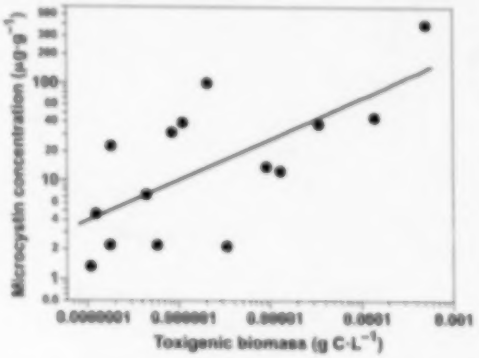
By weighting the biomass of *Anabaena* according to its toxin content relative to that of *Microcystis*, we could derive a measure of total toxigenic biomass in *Microcystis* equivalents. Using this weighted biomass, a positive and significant relationship was observed between microcystin concentration and biomass of toxic cyanobacteria (Fig. 4): microcystin concentration increased as the 0.8 power of biomass, a slope that is not different from 1 (model II, $p = 0.6$). In the lakes surveyed in this study, the microcystin concentration ($\mu g \cdot g^{-1}$) followed the same trend as the biomass of toxic cyanobacteria, depicted as a percentage relative to the total phytoplankton biomass (Fig. 5). Thus, the presence of known toxic species explained most of the toxicity.

The variability of microcystin concentration per unit toxigenic biomass was relatively constant, varying generally 10-fold among lakes, which is not markedly more variable than the carbon/chlorophyll *a* ratio. Much wider ranges were observed for microcystin concentration ($\mu g \cdot L^{-1}$), which varied more than 1000-fold, and for toxigenic cyanobacterial biomass (g C·L^{-1}), which varied by four orders of magnitude among lakes.

Discussion

Our survey showed that all lakes contained detectable levels of toxic cyanobacteria, even the most oligotrophic systems, although usually at concentrations far below levels of concern (Health Canada 2003). This result is in accordance with the current hypothesis that microcystin-producing algae (primarily strains of *Microcystis* and *Anabaena*) contain this toxin as a constitutive physiological component (Hesse et al. 2001; Kaebernick and Neilan 2001). Along the trophic gradient, the only habitat factors that were significantly correlated with water-column toxicity were TP and TN. We will consider the role of each of these factors in turn.

Fig. 4. Correlation plot and regression between log biomass of toxic cyanobacteria and log microcystin concentration in the seston: log microcystin concentration = 3.54 + 0.42 log toxigenic biomass, $r^2 = 0.46$, $p = 0.005$.



Phosphorus is the most limiting nutrient in these lakes (Prairie et al. 2002; Smith and Prairie 2004). In this study there were equally strong correlations between TP and the biomass of total phytoplankton, the biomass of total cyanobacteria alone, and the biomass of toxigenic cyanobacteria, where toxigenic implies microcystin-producing. What is most interesting is that the response to TP of these three variables became increasingly steeper, changing from linear (total phytoplankton) to quadratic (cyanobacteria) to greater than quadratic (toxigenic genera). But there are greater subtleties at work in the appearance of blooms and heavy dominance by cyanobacteria than those due to simple increases in nutrients. Cyanobacteria do not seem to possess affinities or uptake characteristics for phosphorus that are considerably different from those of the eukaryotic algae (Paerl 1996). Like the eukaryotes, they are able to store excess phosphorus in periods of excess supply (luxury consumption). But cyanobacteria are able to control their buoyancy via gas vacuoles, and through rapid vertical movements migrate to phosphate-rich aphotic waters, in this way avoiding phosphate-depleted environments. High TP has frequently been correlated with increased probability of cyanobacterial-bloom events (Canfield et al. 1989; Kotak et al. 2000; Downing et al. 2001). The greater rise in cyanobacterial share of biomass, especially the toxigenic fraction of biomass, relative to the increase in total phytoplankton with an increase in TP could be manifested as a sudden appearance of toxic blooms as lakes are slowly eutrophied by watershed development.

The literature on the effects of phosphorus on microcystin cellular production and content is contradictory. Watanabe and Oishi (1985) showed that under phosphorus-limited conditions, lower toxicity was found in *Microcystis aeruginosa*, probably related to different growth rates. Similarly, Sivonen (1990) found that toxin production in cultures of *Oscillatoria* responded positively to increasing phosphorus at levels between 0.1 and 0.4 mg phosphorus·L^{-1}. However, higher phosphorus concentrations had no further effect on microcystin production. Contrary to the previous findings, Oh et al. (2000) observed that more microcystin per unit dry

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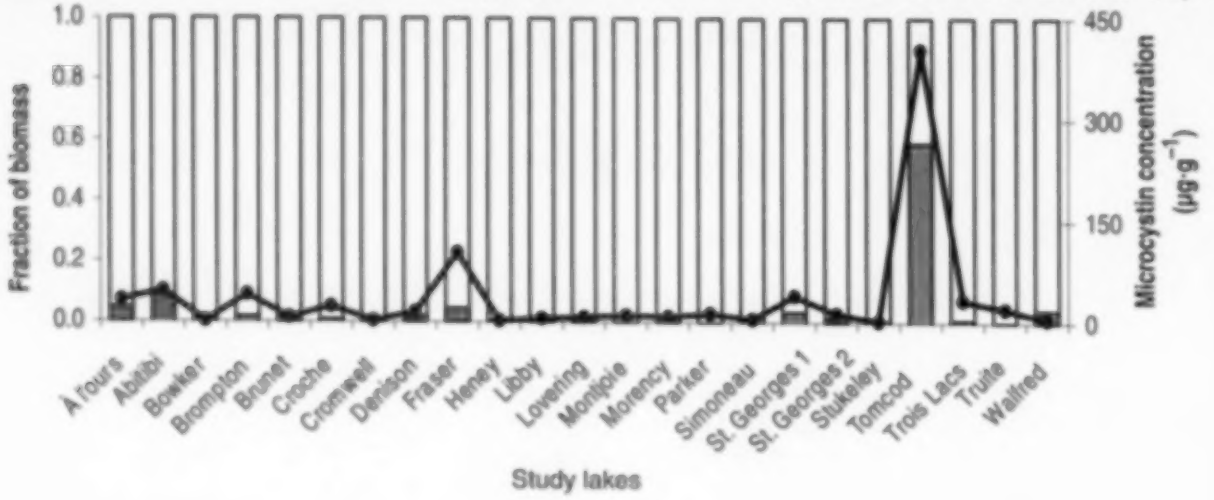
Fig. 5. Percentages of toxic cyanobacteria in the total phytoplankton biomass and microcystin concentrations in the lakes in this study.



weight was produced by cultures of *M. aeruginosa* under more phosphorus-limited conditions. The same authors also found different production ratios of different microcystin variants according to the phosphorus concentrations. For instance, higher ratios of MC-LR to the variant microcystin-RR were found under phosphorus-limited conditions. In the present study, although the general trend was an increase of microcystin cellular content ($\mu g \cdot g^{-1}$ toxic species) with increasing phosphorus concentration, no significant correlation was found. Toxin-producing Cyanobacteria were present in both phosphorus-poor and phosphorus-rich lakes and contained similar cellular levels of toxins in all lakes. This seems to confirm the idea that control of cell-specific microcystin production by phosphorus is of negligible importance among lakes, but that increases in phosphorus act by enhancing the relative contribution of toxigenic Cyanobacteria to total phytoplanktonic biomass. Comparing the concentration of microcystin per litre ($\mu g \cdot L^{-1}$) obtained by Kotak et al. (2000) for a series of lakes in western Canada with our data shows that we detected toxicity at lower phosphorus concentrations, in a range where no observations were recorded in their study. Our results show that toxic cyanobacteria are present and produce toxins in lakes with lower nutrient concentrations. Because they are in low abundance in these environments, the total amount of microcystin is normally below the Canadian ($1.5\ \mu g \cdot L^{-1}$) and World Health Organization ($1\ \mu g \cdot L^{-1}$) guidelines for lifetime drinking-water exposure (Health Canada 2003).

Nitrogen is also an essential element for algal growth. Demands for exogenous nitrogen required for growth and reproduction are consistently high. Nitrogen attenuation and "retention" (often in the form of denitrification) are strongly influenced by hydraulic residence time in an analogous manner to that for phosphorus, and the role of nitrogen in controlling phytoplankton biomass is generally difficult to distinguish from that of phosphorus (Prairie et al. 1989). Because of the atmospheric component that contributes to nitrogen cycles and the possibility of nitrogen fixation by cyanobacteria, few models predicting biomass from nitrogen-loading exist. Yet in this study the observed responses of toxin concentration were stronger to nitrogen content than to TP. Significant correlations were found between TN and microcystin per litre ($\mu g \cdot L^{-1}$) ($r = 0.71$, $p < 0.001$) and per unit weight of net seston ($\mu g \cdot g^{-1}$ dry weight). The first illustrates the importance of nitrogen for the increase in biomass of toxin-producing cyanobacteria. Good correlations between nitrogen and cyanobacterial biomass were also found by Downing et al. (2001), who showed that TN was more strongly correlated with cyanobacterial dominance than TP. The second correlation, however, shows that TN was associated with changing seston content of microcystin, which increased by four orders of magnitude while TN was changing by less than one order of magnitude. In this case, nitrogen availability may be an important factor affecting cellular toxin production, as we might expect for peptides. As discussed below, we believe that when cells are not nutrient-limited, the microcystin content of producing cells does not vary significantly with changes in external nutrient supply. Previous culture studies have shown a consistently greater biomass-specific response to increased nitrogen than to increased phosphorus (Watanabe and Oishi 1985; Codd and Poon 1988; Utkilen and Gjølme 1995). Watanabe and Oishi (1985) reported a remarkable decrease in toxicity when the nitrogen concentration in the medium was reduced, but only minor changes when the phosphorus concentration was lower. Nutrient-ratio experiments by Lee et al. (2000) showed that the microcystin content of *M. aeruginosa* was best correlated with nitrogen content in the growth medium, whether the culture was phosphorus or nitrogen constant. Vezie et al. (2002) found experimentally that high nitrogen concentrations seemed to be necessary to foster growth of both toxic and nontoxic *Microcystis* strains.

The importance of the TN/TP-ratio hypothesis for cyanobacterial dominance has been studied since Smith (1983) suggested that these organisms would be dominant at nitrogen/phosphorus (N/P) ratios below 29. The hypothesis was justified by the idea that cyanobacteria are better competitors for nitrogen than other phytoplankton species. They

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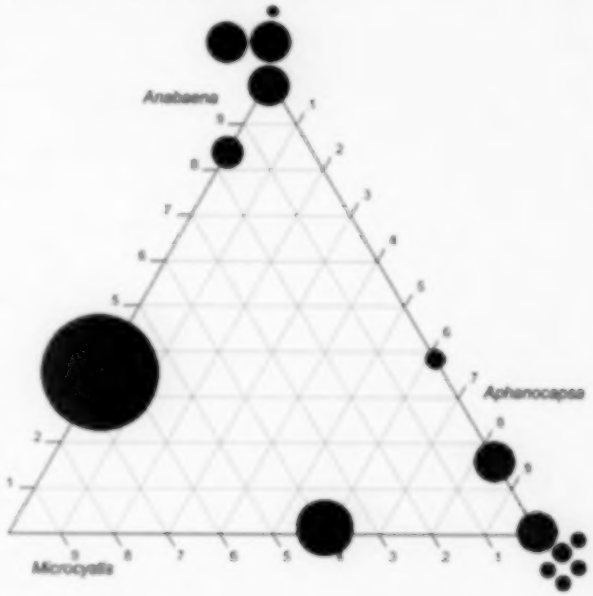
show special features (such as the presence of heterocytes) that allow many taxa of this group to use atmospheric N_2 via nitrogen fixation, thus potentially avoiding situations of nitrogen limitation. They are able to use nitrogen in the form of ammonium, nitrate, and organic nitrogen (urea and a few amino acids). However, other studies have not upheld Smith's hypothesis (Canfield et al. 1989; Vincent 1989; Downing et al. 2001), although Kotak et al. (2000) found that the TN/TP ratio explained most of the variation in microcystin concentration ($\mu g \cdot L^{-1}$). Those authors suggested that a shift in the N/P ratio could increase the incidence of toxic blooms and the production of toxins. In our study no relation was observed between the N/P ratio and lake microcystin concentration, although N/P ratios observed in this study, from 16 to 245, were in a range completely apart from those recorded in the three Alberta lakes, from 1 to 24, that form the basis of Kotak et al.'s (2000) finding. The total amounts of both phosphorus and nitrogen, however, were important to explain the concentration of microcystin ($\mu g \cdot L^{-1}$). Similar to the conclusions of Vincent (1989) and Downing et al. (2001) when explaining the occurrence of cyanobacterial blooms in aquatic ecosystems, we found that the absolute amount of each nutrient, and not their ratio, determined the biomass and toxicity.

In these lakes, the presence and relative abundance of some cyanobacterial genera are the key variables that explain the toxicity of a bloom. The most commonly reported hepatotoxic genera are microplanktonic and include *Anabaena*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Oscillatoria*, *Nostoc*, and *Planktothrix* (Chorus and Bartram 1999), although this list is not exhaustive. Toxicity, or its lack, is not expressed at the generic level, nor at the species level, but varies among strains. Recent reports have also identified microcystin in unicellular and colonial picoplanktonic strains (Domingos et al. 1999). Microcystin is produced by cultured strains of *Aphanocapsa* and *Synechocystis*, for example (Oudra et al. 2002). The production of microcystin by picoplanktonic cyanobacteria opens a new challenge for water-treatment supplies and quality control because of the unsuspected risk of contamination. When we included *Aphanocapsa* as a potential toxic genus in our observations, for instance, we observed a stronger correlation between microcystin concentration ($\mu g \cdot g^{-1}$) and fraction of toxigenic biomass ($r^2 = 0.58$, $p < 0.0001$). In our survey of oligotrophic lakes, *Aphanocapsa* may have been associated with toxicity, especially in those lakes where *Microcystis* and *Anabaena* were not dominant (Fig. 6).

It is still unclear why some cyanobacteria produce toxins. Some may have a regulatory function within cell metabolism (Rapala et al. 1997; Jähnichen et al. 2001). Without knowing the role of the different toxins it is difficult or impossible to interpret the many conflicting reports of controlling environmental factors. Current understanding, however, seems to relate microcystin production to growth. Wicks and Thiel (1990) found a strong correlation between primary production per unit chlorophyll *a* and cell microcystin concentration. They suggest that with increasing light intensity and temperature, both growth rate and toxicity increase, although not at the same rate. Oh et al. (2000) showed a linear relationship between the microcystin-production rate and the cell growth rate, μ. They suggested that the microcystin-production rate in a water system is determined by μ. Orr and Jones (1998) believe that the effect of environmental parameters on microcystin production is the result of their influence on the rate of increase or decline of a strain, but not specific influences on microcystin biosynthetic or catabolic pathways. Lyck (2004) found that microcystin is constitutively produced in a way similar to protein and chlorophyll *a*, implying that just small changes in microcystin cell quota should be expected. Although the minimal and maximal cellular microcystin levels may be modulated by external factors within a toxigenic strain (Sivonen and Jones 1999), and although we may not have identified all toxigenic species and toxic compounds (Pietsch et al. 2001; Chorus 2002), the biomass-specific levels of microcystin are relatively constant and show the same 10-fold range found for carbon:chlorophyll *a* ratios in phytoplankton. This means that the microcystin concentration in all lakes was directly related to the biomass of toxigenic taxa. We conclude that the presence or absence of currently identified toxic species, and the population dynamics of these species, are the principal source of observed changes in bloom toxicity.

Fig. 6. Ternary plot of the relative contributions of the genera *Microcystis* (bottom axis), *Anabaena* (left axis), and *Aphanocapsa* (right axis) to communities associated with a given amount of microcystin in our samples. The circles indicate the amount of toxin per microgram dry weight of seston (i.e., the radius of each circle is directly proportional to the square root of seston toxicity), and their position indicates the unweighted contribution of these three genera to total potential toxigenic biomass. The direction in which the axes should be read is indicated by the small extensions on each axis scale. For example, the largest circle indicates a toxigenic community made up of 65% *Microcystis*, 35% *Anabaena*, and 0% *Aphanocapsa*. Circles on the vertices or outside the triangle represent communities made up entirely of the cyanobacterial genus indicated at that vertex.



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Branching complexity and morphological characteristics of coarse woody structure as lacustrine fish habitat

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Abstract: The objective of this study was to quantify the physical characteristics of coarse woody structure (CWS) as fish habitat in a north temperate lake. Sixteen species of fish were observed in submerged CWS habitat. Branching complexity, distance above the bole, area below the bole, distance to other CWS, and water depth around CWS were significantly related to abundance of schooling cyprinids (Cyprinidae), rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*), bluegill (*Lepomis macrochirus*), yellow perch (*Perca flavescens*), and walleye (*Sander vitreus*). Branching complexity was the most common characteristic of CWS related to richness, diversity, and total adult abundance of fish taxa, but was not correlated with the total lengths of fish found in submerged trees. Branching-complexity values ranged from 1 (simple) to 500 (moderately complex) in the littoral zone; for comparison, a living riparian conifer had a branching-complexity value of over 1000. Most CWS in the littoral zone was composed of simple trees without branching, but fish tended to inhabit CWS with branching-complexity values greater than 45. This study shows the importance of CWS with fine branching as littoral-zone fish habitat.

Résumé : L'objectif de notre travail est de décrire quantitativement les caractéristiques physiques des structures ligneuses grossières (CWS) qui servent d'habitat aux poissons dans un lac tempéré nordique. Seize espèces de poissons s'observent dans l'habitat CWS. La complexité des branchements, la distance au-dessus du tronc, la surface sous le tronc, la distance des autres CWS et la profondeur de l'eau autour du CWS sont toutes reliées à l'abondance des bancs de cyprinidés (Cyprinidae), de crapets de roche (*Ambloplites rupestris*), d'achigans à petite bouche (*Micropterus dolomieu*), de crapets arlequins (*Lepomis macrochirus*), de perchaudes (*Perca flavescens*) et de dorés jaunes (*Sander vitreus*). La complexité des branchements est la caractéristique des CWS qui est le plus souvent associée à la richesse en taxons des poissons, à leur diversité et à l'abondance totale des adultes, mais elle n'est pas reliée aux longueurs totales des poissons trouvés parmi les arbres submergés. Les valeurs de complexité des branchements varient de simple (1) à modérément complexe (500) dans la zone littorale; pour fins de comparaison, un conifère vivant sur la berge possède une valeur de complexité des branchements de plus de 1000. La plupart des CWS dans la zone littorale consistent en arbres simples sans branches, alors que les poissons tendent à s'abriter dans des CWS avec des valeurs de complexité des branchements supérieures à 45. Notre étude démontre l'importance des CWS avec un branchement élaboré comme un habitat pour les poissons dans la zone littorale.

[Traduit par la Rédaction]

Introduction

Our ecological understanding of coarse woody structure (CWS) in lacustrine systems is based primarily, albeit incompletely, on studies of wood in fluvial systems (Andrus et al. 1988; Bilby and Ward 1991; Maser and Sedell 1994). However, there are only general understandings of how CWS functions as lacustrine fish habitat. For example, it has been presumed that CWS in the littoral zone provides fish with ambush habitat and a refuge from predation (Tabour and Wurtsbaugh 1991; Everett and Ruiz 1993; Biro and Ridgway 1995). Nevertheless, fish generally are attracted to physical structure and thus complex habitats (Moring et al. 1989; Rogers and Bergersen 1999). Moring et al. (1989) showed that concentrations of pulpwood in a Maine reservoir attracted longnose suckers (*Catostomus catostomus*), white sucker (*Catostomus commersonii*), and golden shiner (*Notemigonus crysoleucas*). Rogers and Bergersen (1999) found the placement of synthetic physical structures into two Colorado reservoirs attracted northern pike (*Esox lucius*),

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largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and white crappie (*Pomoxis annularis*).

CWS, macrophytes, and substrate of different particle sizes are natural contributors to fish habitat in littoral zones, but can be difficult to quantify. Many studies of habitat complexity have focused primarily on responses of fish distributions to density of aquatic macrophytes (Savino and Stein 1989; Bryan and Scarnecchia 1992; Eklöv 1997). Others have dealt with the abundance of logs (Eklöv 1997) or the surface area of coarse wood per unit area in lakes (Wallace and Benke 1984). These studies did not quantify the morphology of trees and therefore could not distinguish between different types of CWS as fish habitat (i.e., between boles with fine branching and boles without branches). Because trees and their structure determine the dimensions of habitat complexity in the littoral zone, methods need to be developed for describing and quantifying the branching complexity (i.e., fine branching) and other morphological attributes of coarse woody habitat in relation to fish communities. If fish prefer structurally complex habitats, as is suggested by studies of macrophytes, then fish may be more numerous around CWS with greater amounts of fine branching.

CWS recruitment into lakes is regulated by forest succession, stand dynamics, and anthropogenic alterations, although catastrophic events (e.g., blowdown, fire) can accelerate recruitment (Guyette and Cole 1999). Recently recruited CWS can provide more complex branching and perhaps fish habitat (i.e., medium and fine branching or leaves) than older littoral-zone trees, which have been subjected to movement, deterioration, and decomposition by wind–wave activity, ice scouring, and natural decay. As a result, simple boles without branches may dominate lake shorelines, particularly when no recent recruitment of complex CWS has occurred along tree-harvested shorelines (Guyette and Cole 1999). In addition, removal of trees in riparian areas and littoral zones has further altered littoral-zone habitats (Christensen et al. 1996; Guyette and Cole 1999; Scully et al. 2000). If morphological characteristics of CWS are important to fish communities, and morphological characteristics change over time, then the relationships between morphological structure and fish community characteristics (i.e., species richness, species abundance, fish total length (TL), etc.) should be correlated and provide insight into lacustrine ecology and the influence of riparian forest management.

Materials and methods

Study area

This study was conducted on Katherine Lake (45.8050°N, 89.7115°W), Oneida County, Wisconsin, an oligotrophic lake with a surface area of 238 ha and a total shoreline of 15.6 km. The maximum depth is 9.7 m with 11.5% of the lake under 1 m deep and 50.5% between 1 and 6 m deep. The littoral-zone substrate is composed of sand (55%), gravel (20%), and rubble (15%) with some boulders and muck. The fishes of Katherine Lake include muskellunge (*Esox masquinongy*), cisco (*Coregonus artedi*), black bullhead (*Ameiurus melas*), yellow bullhead (*Ameiurus natalis*), white sucker, bluntnose minnow (*Pimephales notatus*), blacknose shiner (*Notropis heterolepis*), burbot (*Lota lota*), mottled sculpin (*Cottus bairdii*), walleye (*Sander vitreus*), yellow perch (*Perca flavescens*), johnny darter (*Etheostoma nigrum*), Iowa darter (*Etheostoma exile*), largemouth bass, smallmouth bass (*Micropterus dolomieu*), black crappie (*Pomoxis nigromaculatus*), rock bass (*Ambloplites rupestris*), pumpkinseed (*Lepomis gibbosus*), and bluegill. The lake has both undeveloped and developed (i.e., houses) shoreline. The shoreline is predominantly upland (95%), but also has limited wetlands of shrub and coniferous flora. The riparian area is primarily composed of white pine (*Pinus strobus*), red pine (*Pinus resinosa*), balsam fir (*Abies balsamea*), paper birch (*Betula papyrifera*), and white oak (*Quercus alba*).

Study sites and habitat variables

Characteristics of submerged trees and respective littoral-zone habitat were quantified at 63 sites in Katherine Lake using random-stratified sampling. Initially, 30 randomly selected sites were evaluated to assess characteristics of CWS and their distribution in the littoral zone. An additional 33 sites were added using random-stratified sampling to insure that highly complex trees were adequately sampled to evaluate relationships between branching complexity and fish. Aquatic sites were classified as with or without CWS, and CWS was classified as either coniferous or deciduous. Sites were also classified into seven levels based on the branching complexity of CWS present, with a target sample size of approximately 10 sites per complexity class. Categories included sites having no wood (i.e., zero branching complexity, $n = 10$), sites with coniferous CWS with branching complexities of 1–99 ($n = 10$), 100–199 ($n = 8$), and >200 ($n = 7$), and deciduous CWS with branching complexities of 1–49 ($n = 12$), 50–99 ($n = 8$), and >100 ($n = 8$). The branching complexities of coniferous and deciduous CWS varied because of differences between whorled and dichotomous branching patterns (Newbrey 2002). Of the 53 sites with CWS, 25 were classified as coniferous and 28 were considered deciduous.

Transects were set perpendicular to shore and all CWS was inventoried within 3 m either side of the transect line from shore to 3 m depth. Transects were placed a minimum of 60 m from other transects to reduce spatial autocorrelation of fish–CWS relationships (Platts et al. 1983). If a selected transect fell on a site that was developed (i.e., a house built on a typical 30 m wide lot), then the transect was positioned such that the 6 m wide sampling area was centered within the developed property boundary so that all data collected represented the same land use. We inventoried all trees at all sites to assess the distribution of branching complexity in the littoral zone. Conifers (e.g., white pine, red pine, tamarack (*Larix laricina*), eastern hemlock (*Tsuga canadensis*), and balsam fir) were identified as having whorled branching patterns as opposed to the relatively dichotomous branching patterns of angiosperms, referred to as deciduous trees. CWS was defined as any entirely or partially submerged tree ≥10 cm in diameter anywhere along its length (e.g., Christensen et al. 1996; McHenry et al. 1998). Trees were included in the study site if any part of the CWS bole was located within the 6 m wide sampling area. Each piece of CWS received a numbered aluminum tag, and the morphology, location, orientation, and site characteristics were quantified (Newbrey 2002) (Table 1).

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Table 1. Variables used to examine relationships between characteristics of submerged trees and associated littoral-zone habitat with regard to fish in Katherine Lake, Oneida County, Wisconsin.

Predictor variable	Response variable
CWS characteristics	Adult taxon richness
Branching complexity	Adult diversity without cyprinids
Bole diameter (cm)	Adult abundance without cyprinids
Freeboard (m)	Fish abundance
Clearance (m^2)	Fish total length (cm)
Wet length (m)	
Bridging distance (m)	
Distance to other CWS (m)	
Minimum depth of tree (m)	
Total depth at tree (m)	
Site characteristics	
Site slope	
Mean depth at tree (m)	
Dominant substrate	
Subdominant substrate	

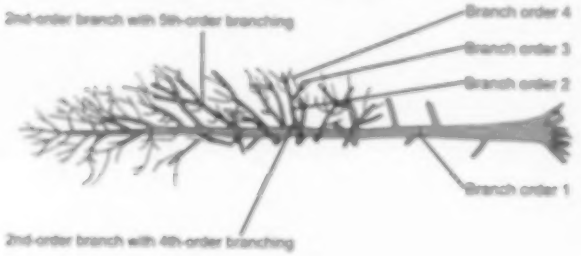
Note: CWS, course woody structure.

We evaluated the fish habitat provided by each piece of CWS by quantifying the branching present. More specifically, a branching-complexity index was defined as the sum of individual branching values of one or more segments of basal branching for each piece of CWS (i.e., the sum of second-forked branches from the bole or segments of a tree):

$$\text{Complexity} = \sum_{s=1}^{s=1}\sum_{f=1}^{f=1}(v_f n_i) + \sum_{f=1}^{f=1}(v_f n_i) + \ldots + \sum_{f=1}^{f=1}(v_f n_i)$$

where s is a given basal segment, x is a segment in a series of segments, f is a family of branching in a segment, j is a branch in a series of branches in a family of 2nd-order branching in a segment, v is the classification value of a family of 2nd-order branching in a segment, n is the total number of branches in a family of 2nd-order branching in a segment, and i is the maximum (up to 5) order of branching attached to a 2nd-order branch in a segment. Basal branching consisted of large branches stemming from the bole. Each segment's branching complexity was calculated by classifying tree branches into discrete branching families based on the number of times (branch order) a given branch had forked from the bole. A tree segment was defined as the bole or each half of any number of dichotomous splits of basal branching where each branch was greater than 10 cm in diameter. A dichotomous split was defined as a point where basal branches divide into branches of approximately equal diameter (i.e., all branches must be within 15% of the diameter of each other). A tree may have several sets of dichotomous splits in basal branching, and each segment between the dichotomous branching points may contribute significantly to crown volume. Such branching is more typical of deciduous trees but may occur in conifers when leaders develop after a terminal leader is damaged. Complexity was calculated for each segment and then all segments were summed (Newbrey 2002). Branching order was assigned by applying a modified classification system using inverted stream order (Strahler 1957) to the branching of wood and

Fig. 1. Conifer showing branching orders 1–5. A branching point originating from a branch of order 2 or greater was defined as either (*i*) a 50/50 split in branching or (*ii*) a branch ≥15 cm long originating from the main axis. Branching complexity was quantified by determining a complexity value for each branch and then summing values for all branches. This hypothetical tree has a branching complexity of approximately 125.



then tallying the total number of branches in each order. The bole or largest central stem was assigned an order of 1, all branches extending from the bole were assigned an order of 2, all branches extending off order-2 branches were designated order 3, etc. (Fig. 1) (Newbrey 2002). A branch was defined as lignified wood a minimum of 15 cm in length. A Y-shaped branching point originating from a branch of order 2 or greater denoted two new branches and a new branch order if the parent branch had split (visual estimation) about 50/50 (±3%) into two branches. If any non-attached branch (≥15 cm in length) was touching any part of the tree, the branch was included in the branch count as if it were attached for description as fish habitat; however, it was noted as unattached for description of branching complexity of randomly selected CWS. Branches were quantified to 1 m above the water surface. If a branch extended out of the water but bent back into the water, it was counted as submerged. The tree with the greatest branching complexity at each site was selected for individual tree analysis. Our algorithm will produce branching-complexity values from 1 to infinity. For reference, however, a 24.8 m tall white pine with needles had a branching complexity of 1094 and weathered littoral-zone CWS had a maximum value of about 500.

In addition to branching complexity, we measured eight other CWS characteristics. Bole diameter (m) was a single measurement of the largest diameter above the root wad. Freeboard was the distance (m) from the deepest part of the bole to the surface of the water. Clearance for a single tree was calculated by summing individual clearance segments if more than one portion of the bole rose above the lake substrate. Each clearance segment was measured as the distance in a vertical plane from underneath the bole to the lake bottom multiplied by the length of uninterrupted, open length distance. Clearance and freeboard were measured from the bole and not from branches attached to the bole. Wet length was the length (m) of the bole in water. Bridging distance was the length (m) of the bole suspended above the water and was measured along the water and under the bole from the point at which the bole entered the water back to the shoreline–water interface. The nearest distance (m) to other CWS up to 30 m away was recorded. Minimum water depth

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(m) was measured at the shallowest water in contact with the bole (e.g., branch order 1). Total water depth (m) at the tree was the maximum depth of water in contact with the bole or any branch.

Other habitat characteristics were also quantified at each site, including mean water depth, site slope, and dominant and subdominant substrates. Characteristics were measured at four equidistant points along five equidistant transects perpendicular to shore out to a depth of 3 m. Substrate composition was visually estimated from grids using a modified substrate-particle scale (Wentworth 1922; Platts et al. 1983). Mean water depth (m) and mean site slope (rise over run) were also calculated.

Fish

Fish variables were quantified at each piece of CWS a minimum of 24 h after habitat was quantified at the site to allow displaced fish to reposition within the tree. Richness, diversity, and abundance of fish taxa were determined and fish TL was measured at each site on two separate occasions, once while SCUBA diving and once while snorkeling. SCUBA diving allowed for deep observations and snorkeling yielded shallow observations. The two observations were made on separate days for 20 min·day^{-1} at each site. All observations were conducted by a single diver to minimize observer error and disturbance (Lonzarich et al. 1998). Fish variables were quantified only on sunny days between the hours of 0900 and 1700 from 15 June to 15 August in 2000 and 2001. Only the maximum number of each species observed at each stop along a tree was recorded during visual surveys to insure that fish were not recorded more than once. Thus, observed fish provided an index of abundance rather than absolute abundance. The mean of the maximum numbers of individuals of all species observed during the two surveys was calculated to conservatively represent the number of fish on a tree. Fish were included in the survey if they were within 1 m of any part of a tree's bole or its branches. Three sites that had trees with needles or leaves were excluded from the study because needles and leaves presented a visual barrier to counting fish. Fish species diversity was calculated using Shannon's diversity index (see Zar 1999, p. 41, eq. 4.17) and did not include cyprinids, which could not be identified to species during diver observations. Fish TL was estimated with a ruler inscribed on the data slates by measuring from an observed point (previous fish location) along a branch or bole. The estimated TL was then applied to other fish in close proximity (within 0.5 m of the measured fish) that appeared to be approximately the same size.

Data analyses

Differences in taxon richness, diversity, and adult abundance of fish were compared between sites with and without CWS using a Mann–Whitney *U* test. Differences were significant at an adjusted α value ($P \leq 0.005$) using a Holm simultaneous testing procedure, which adjusts α when multiple tests are employed (Neter et al. 1996). Alpha was adjusted for each family test of the analyses of conifer and deciduous trees and the most conservative α value was selected among the pairs of family tests. Linear and multiple linear regressions were used to ascertain relationships between fish and habitat characteristics. We used forward stepwise regression with α (0.05) as variable selection criteria. Young-of-the-year fish were not included in these analyses because of the variability in hatching times among species. In all analyses, we used the tree of greatest complexity at each site and the fish associated with that tree. Cyprinids were treated as one taxon to determine taxon richness because schooling cyprinids can be difficult to identify under water and were not included in diversity and adult-abundance analyses. We examined the distribution of the untransformed residuals for all variables and 12 showed patterns problematic for analysis. A natural-log transformation improved the distribution of the residuals for the 12 variables. Freeboard, clearance, distance to other CWS, taxon richness, diversity, and fish abundance were natural-log-transformed ($\ln(x + 1)$); branching complexity, bole diameter, wet length, dominant substrate, subdominant substrate, and fish TL were natural-log-transformed (ln) to linearize the data and randomize the distribution of the residuals. Those variables not transformed included bridging distance, minimum water depth, total depth at the tree, site slope, and mean depth at the tree. Alpha was again adjusted for the number of regression models run for each test using a Holm simultaneous testing procedure (Neter et al. 1996). All tests were performed with SYSTAT® version 10.0 (Systat Software Inc. 2002).

Results

Randomly sampled CWS in the littoral zone of Katherine Lake was dominated (>80%) by short ($\bar{x}$ = 2.5 m long), simple, small-diameter coniferous boles (Fig. 2). Both coniferous and deciduous trees with no clearance (i.e., the entire length of the bole was in contact with the substrate) were more numerous (>60%) than trees with clearance. Boles were generally positioned close to the surface (i.e., small freeboard distance) because they were in shallow water (Fig. 2). Bridging distance was zero for most coniferous and deciduous trees (Fig. 2).

Random sampling did not permit description of the full range of CWS branching complexities. The CWS located via random-stratified sampling consisted of trees of greater branching complexity for both groups; this complexity represented an uncommon condition in contrast to the moderately common, structurally simple, randomly selected trees (Fig. 3).

The presence of CWS significantly affected only four of the spatial patterns of fishes in the littoral zone of Katherine Lake. Littoral-zone sites with coniferous CWS had a significantly ($P \leq 0.005$) greater abundance of smallmouth bass and bluegill than sites without wood; however, other fish taxa were not significantly more abundant at wooded sites (Table 2). Schooling cyprinids, walleye, and bluegill were more abundant in coniferous than in deciduous trees. Some species, such as muskellunge, were rare at both coniferous and deciduous sites, thus precluding analysis, but tended to be more abundant at some wooded sites. Black crappie were rare at coniferous sites and walleye were rare at deciduous sites, thus precluding analysis, but overall were still more common at wooded sites than at non-wooded sites (Table 2). Furthermore, 10 species were not included in any of the analyses because they were rarely encountered or cryptic in

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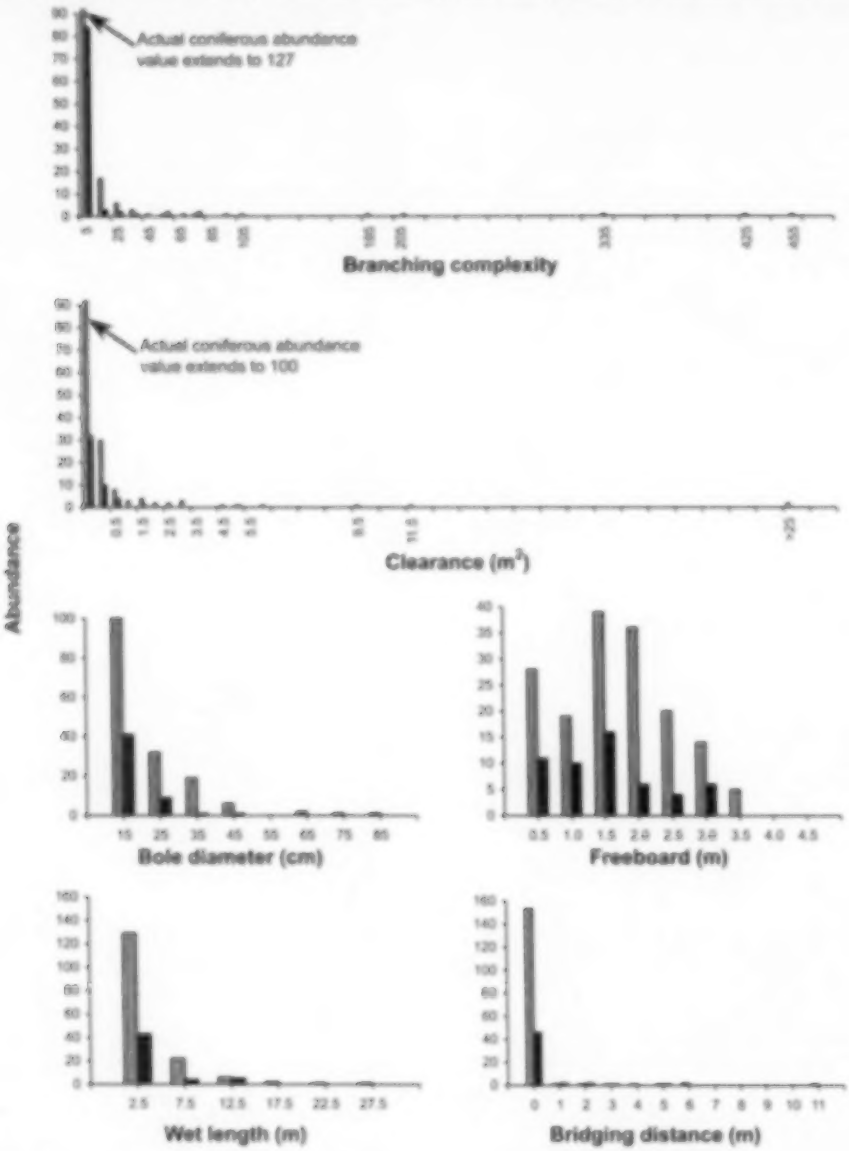
Fig. 2. Abundance of structural characteristics of randomly selected coniferous (shaded bars) and deciduous (solid bars) coarse woody structure (CWS) in Katherine Lake, Oneida County, Wisconsin. Branching-complexity values only include branches attached to the tree.



nature: cisco, black bullhead, yellow bullhead, white sucker, burbot, mottled sculpin, johnny darter, Iowa darter, largemouth bass, and pumpkinseed.

Branching complexity was the most common (i.e., 16 of 22 models), and often the most descriptive, CWS characteristic explaining variation in fish taxon richness, diversity, total adult abundance (all taxa combined), and the abundance of eight taxa for coniferous and deciduous trees (Tables 3–5). Taxon richness, diversity, and total adult abundance were higher at sites where wood had greater branching complexity, as were abundance of six fish taxa for conifers and four taxa for deciduous trees (Table 3; Figs. 4–6). Branching complexity was significantly and positively related to all fish measures. Few to no fish were generally found at coniferous and deciduous branching complexities of less than 45 (ln branching complexity = 3.8), except walleye and yellow perch, which appeared to generally increase after branching complexity reached 170 (ln branching complexity = 5.1) (Figs. 4, 5). For most taxa in deciduous trees, fish metrics increased with branching complexity; significant relationships were found for taxon richness, diversity, total adult abundance, and abundance of cyprinids, smallmouth bass, black crappie, rock bass, and bluegill.

Other characteristics of trees also influenced the distribution and TLs of fish (for additional detail see Newbrey 2002). For example, taxon richness, diversity, and adult

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Fig. 3. Abundance of branching-complexity characteristics of (*a*) coniferous and (*b*) deciduous CWS used in data analysis. Sites were selected in random (open bars) and random-stratified (solid bars) manners in Katherine Lake. Recently deposited trees with needles were excluded from analysis. Branching-complexity values include branches not attached to the tree but touching the tree in order to describe the branching characteristics in terms of fish habitat.

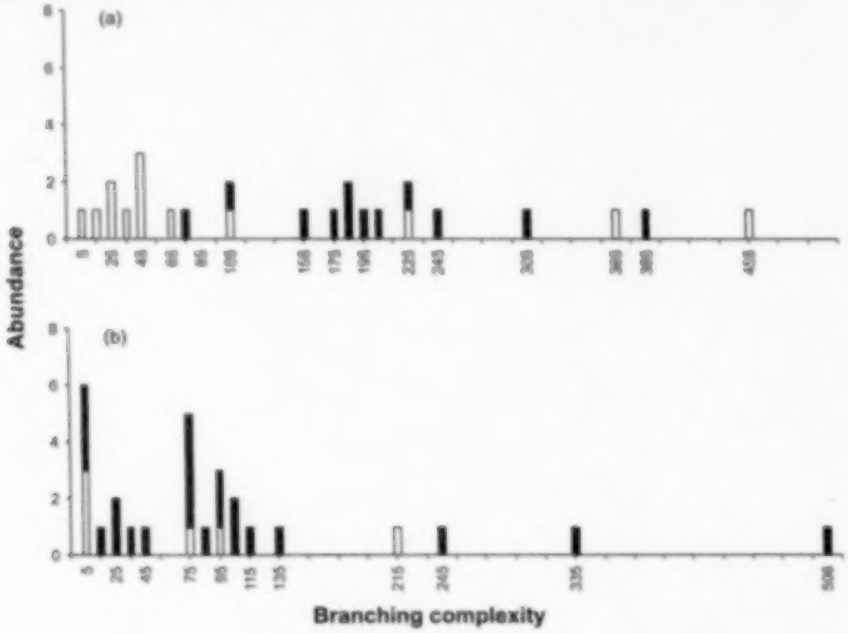

Table 2. Mean taxon richness, diversity (Shannon's diversity index), adult abundance (without cyprinids), and abundance of eight species of fish at sites with CWS and without CWS (No CWS) (n = 10) based on Mann–Whitney U tests, and fish metrics from sites having coniferous (n = 25) versus deciduous (n = 28) trees.

				No CWS vs. coniferous trees		No CWS vs. deciduous trees		Coniferous trees vs. deciduous trees	
Variable	No CWS (mean ± 1 SE)	Coniferous trees (mean ± 1 SE)	Deciduous trees (mean ± 1 SE)	U	P	U	P	U	P
Taxon richness	3.9±0.41	4.8±0.71	3.0±0.48	104.5	0.450	187.5	0.112	456.0	0.057
Diversity	0.1±0.05	0.3±0.05	0.2±0.04	62.0	0.018	94.5	0.119	401.5	0.352
Adult abundance	2.2±1.13	42.2±9.77	8.3±2.83	61.5	0.020	92.0	0.109	480.0	0.020
Muskellunge abundance	0.0±0.00	0.3±0.09	0.1±0.03	85.0	0.047	125.0	0.287	413.0	0.071
Cyprinid abundance	13.3±6.15	771.0±179.37	129.8±80.37	65.0	0.027	166.0	0.328	516.5	**0.001**
Walleye abundance	0.0±0.00	7.9±2.81	0.3±0.27	65.0	0.010	130.0	0.392	500.5	**0.001**
Yellow perch abundance	0.0±0.00	1.9±0.74	0.3±0.17	75.0	0.022	115.0	0.158	439.5	0.045
Smallmouth bass abundance	0.2±0.11	3.0±0.55	1.2±0.32	14.0	**<0.001**	66.0	0.010	467.0	0.031
Black crappie abundance	0.0±0.00	1.2±0.68	0.2±0.12	110.0	0.259	120.0	0.213	348.0	0.952
Rock bass abundance	0.1±0.10	4.9±1.92	1.1±0.45	56.5	0.006	89.5	0.052	420.0	0.171
Bluegill abundance	1.0±0.95	26.8±5.77	4.3±1.98	41.0	**0.002**	115.5	0.325	499.0	**0.004**

Note: Fish data from sites with CWS were taken from the tree of greatest branching complexity as opposed to sites without CWS, where all fish were counted at each site. The α value for each model was set at $P \leq 0.005$ using a Holm simultaneous testing procedure. Values in boldface type are significantly different.

abundance were also described by other CWS characteristics such as freeboard (two conifer models, two deciduous models), bridging distance (three conifer models), distance to other CWS (two conifer models), and bole diameter (two deciduous models). Fish TL was also correlated with CWS characteristics (Table 6). Freeboard in conifers, which ranged from 0.2 to 4.4 m ($\bar{x}$ = 1.5 m), was positively correlated with smallmouth bass TL and negatively related to diversity, muskellunge abundance, and bluegill TL. Freeboard in deciduous trees, which ranged from 0.0 to 2.7 m ($\bar{x}$ = 1.0 m), was positively related in two models and negatively related in three models. Bridging distance, i.e, the distance of the bole over the water, ranged from 0.0 to 6.4 m ($\bar{x}$ = 1.2 m) in conifers and was positively correlated in five mod-

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Table 3. Relationships between taxon richness, diversity (Shannon's diversity index), and fish abundance and CWS branching complexity.

Variable	Regression equation	*n*	SEE	r^2	*P*
Coniferous					
Taxon richness	= 0.666 × ln complexity – 1.575	20	0.448	0.748	**<0.001**
Diversity	= 0.122 × ln complexity – 0.308	18	0.136	0.520	**<0.001**
Adult abundance	= 1.459 × ln complexity – 4.055	20	0.874	0.789	**<0.001**
Muskellunge abundance	= 0.080 × ln complexity – 0.214	6	0.282	0.097	0.130
Cyprinid abundance	= 2.432 × ln complexity – 7.220	16	2.124	0.638	**<0.001**
Walleye abundance	= 0.827 × ln complexity – 2.700	12	1.111	0.427	**<0.001**
Yellow perch abundance	= 0.440 × ln complexity – 1.427	10	0.727	0.330	**0.003**
Smallmouth bass abundance	= 0.391 × ln complexity – 0.886	20	0.618	0.350	**0.002**
Black crappie abundance	= 0.202 × ln complexity – 0.644	3	0.774	0.084	0.160
Rock bass abundance	= 0.535 × ln complexity – 1.600	13	0.969	0.291	**0.005**
Bluegill abundance	= 1.303 × ln complexity – 3.903	16	1.070	0.666	**<0.001**
Deciduous					
Taxon richness	= 0.379 × ln complexity – 0.240	24	0.382	0.724	**<0.001**
Diversity	= 0.084 × ln complexity – 0.098	20	0.124	0.549	**<0.001**
Adult abundance	= 0.635 × ln complexity – 0.877	22	0.642	0.722	**<0.001**
Muskellunge abundance	= 0.023 × ln complexity – 0.056	2	0.102	0.12	0.071
Cyprinid abundance	= 0.605 × ln complexity – 1.176	6	2.106	0.180	0.024
Walleye abundance	= 0.086 × ln complexity – 0.225	2	0.392	0.113	0.080
Yellow perch abundance	= 0.102 × ln complexity – 0.218	5	0.371	0.167	0.031
Smallmouth bass abundance	= 0.222 × ln complexity – 0.312	14	0.562	0.293	**0.003**
Black crappie abundance	= 0.096 × ln complexity – 0.242	4	0.285	0.232	**0.009**
Rock bass abundance	= 0.245 × ln complexity – 0.524	10	0.558	0.340	**0.001**
Bluegill abundance	= 0.467 × ln complexity – 1.015	10	0.916	0.409	**<0.001**

Note: Taxon richness includes all cyprinids as a single taxon, but diversity and adult abundance exclude them. Values for fish taxon richness, diversity, adult abundance, and abundance of each species were natural-log-transformed (ln) and summed with a value of 1 prior to transformation; *n* is the sample size; SEE, standard error of the estimate; the α value for each model was set at $P \leq 0.017$ using a Holm simultaneous testing procedure. Values in boldface type denote significant relationships.

Table 4. Total adult taxon richness, diversity (Shannon's diversity index), and total adult abundance as functions of CWS morphological characteristics and site characteristics.

Variable	Coefficient	$P_{variable}$	Intercept	*n*	SEE	r^2	P_{model}
Coniferous							
Taxon richness							
Complexity (ln)	0.599	<0.001	–1.087	20	0.402	0.806	**<0.001**
Distance to other CWS (ln(*x* +1))	–0.408	0.017					
Diversity							
Complexity (ln)	0.091	<0.001	–0.022	18	0.108	0.682	**<0.001**
Freeboard (ln(*x* +1))	–0.182	0.001					
Adult abundance							
Complexity (ln)	1.338	<0.001	–3.796	20	0.733	0.845	**<0.001**
Bridging distance	0.275	0.003					
Deciduous							
Taxon richness							
Complexity (ln)	0.379	<0.001	–0.240	24	0.382	0.724	**<0.001**
Diversity							
Complexity (ln)	0.062	<0.001	–0.122	20	0.097	0.711	**<0.001**
Bole diameter (ln)	0.007	0.001					
Freeboard (ln(*x* +1))	–0.160	0.009					
Adult abundance							
Site slope	1.172	0.030	–2.655	22	0.595	0.752	**<0.001**
Complexity (ln)	0.668	<0.001					

Note: Taxon richness includes cyprinids as a single taxon, but diversity and adult abundance do not. Values for fish taxon richness, diversity, and adult abundance were natural-log-transformed (ln) and summed with a value of 1 prior to transformation; the α value was set at 0.05 for selection of each variable ($P_{variable}$); *n*, sample size; SEE, standard error of the estimate; r^2, adjusted r^2 in all cases; the α value for each test (P_{model}) was set at $P \leq 0.013$ using a Holm simultaneous testing procedure. Values in boldface type denote significant relationships.

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Table 5. Fish abundance as a function of coniferous and deciduous morphological and site characteristics.

Variable	Coefficient	$P_{variable}$	Intercept	n	SEE	r^2	P_{model}
Coniferous							
Muskellunge abundance							
Freeboard (ln(x + 1))	–0.276	0.009	0.305	6	0.232	0.361	**0.003**
Bridging distance	0.063	0.023					
Cyprinid abundance							
Complexity (ln)	2.135	<0.001	–5.042	16	1.936	0.686	**<0.001**
Distance to other CWS (ln(x + 1))	–1.820	0.026					
Walleye abundance							
Complexity (ln)	0.827	<0.001	–2.700	12	1.111	0.427	**<0.001**
Yellow perch abundance							
Complexity (ln)	0.440	0.003	–1.427	10	0.727	0.330	**0.003**
Smallmouth bass abundance							
Complexity (ln)	0.391	0.002	–0.886	20	0.618	0.322	**0.002**
Black crappie abundance							
No relation	—	—	—	3	—	—	—
Rock bass abundance							
Complexity (ln)	0.535	0.005	–1.600	13	0.969	0.291	**0.005**
Bluegill abundance							
Complexity (ln)	1.183	<0.001	–3.646	16	0.969	0.714	**<0.001**
Bridging distance	0.273	0.022					
Deciduous							
Muskellunge abundance							
No relation	—	—	—	2	—	—	—
Cyprinid abundance							
Complexity (ln)	0.605	0.024	–1.176	6	2.106	0.149	0.024
Walleye abundance							
No relation	—	—	—	2	—	—	—
Yellow perch abundance							
Complexity (ln)	0.102	0.031	–0.218	5	0.371	0.135	0.031
Smallmouth bass abundance							
Complexity (ln)	0.161	0.022	–0.709	14	0.513	0.389	**0.001**
Bole diameter (ln)	0.023	0.019					
Black crappie abundance							
Complexity (ln)	0.096	0.009	–0.242	4	0.285	0.232	**0.009**
Rock bass abundance							
Site slope	1.836	0.003	–2.075	10	0.452	0.550	**<0.001**
Complexity (ln)	0.276	<0.001					
Freeboard (ln(x + 1))	–0.538	0.035					
Bluegill abundance							
Site slope	2.919	0.008	–4.047	10	0.810	0.520	**<0.001**
Complexity (ln)	0.524	<0.001					

Note: All fish abundance values were natural-log-transformed (ln) and summed with a value of 1 prior to transformation; the α value was set at 0.05 for selection of each variable ($P_{variable}$); n, sample size; SEE, standard error of the estimate; r^2, adjusted r^2 in all cases; the α value for each test (P_{model}) was set at $P \leq 0.013$ using a Holm simultaneous testing procedure. Values in boldface type denote significant relationships.

els. Bridging distance for deciduous trees, which ranged from 0.0 to 4.5 m ($\bar{x}$ = 1.2 m), was positively related to smallmouth bass TL. Distance of coniferous CWS to other CWS ranged from 0.0 to 4.0 m ($\bar{x}$ = 0.8 m) and was positively and negatively related in two models each. Distance from deciduous CWS to other CWS ranged from 0.0 to 6.0 m ($\bar{x}$ = 1.0 m) and was positively related to smallmouth bass TL, as in conifers.

Conifer bole diameter, which ranged from 10 to 73 cm ($\bar{x}$ = 40 cm), was not used in the analyses because it was highly correlated with branching complexity (R = 0.706) (Newbrey 2002). Deciduous tree bole diameter, which ranged from 13 to 65 cm ($\bar{x}$ = 27 cm), was positively correlated with diversity and smallmouth bass abundance. The remaining two coniferous and deciduous CWS variables, clearance and wet length, were moderately to highly correlated with branching complexity (R values ranged from 0.48 to 0.80) (Newbrey 2002).

Site characteristics were not as commonly related to fish distribution as were CWS attributes. Site slope, mean depth, and dominant and subdominant substrates were significant for some species, especially when correlated with fish TL (i.e., rock bass and bluegill in Table 6). Dominant substrates ranged from <0.2 mm (fine organic particles and silt) to 76.1–149.9 mm (cobble), the most commonly encountered substrate being sand.

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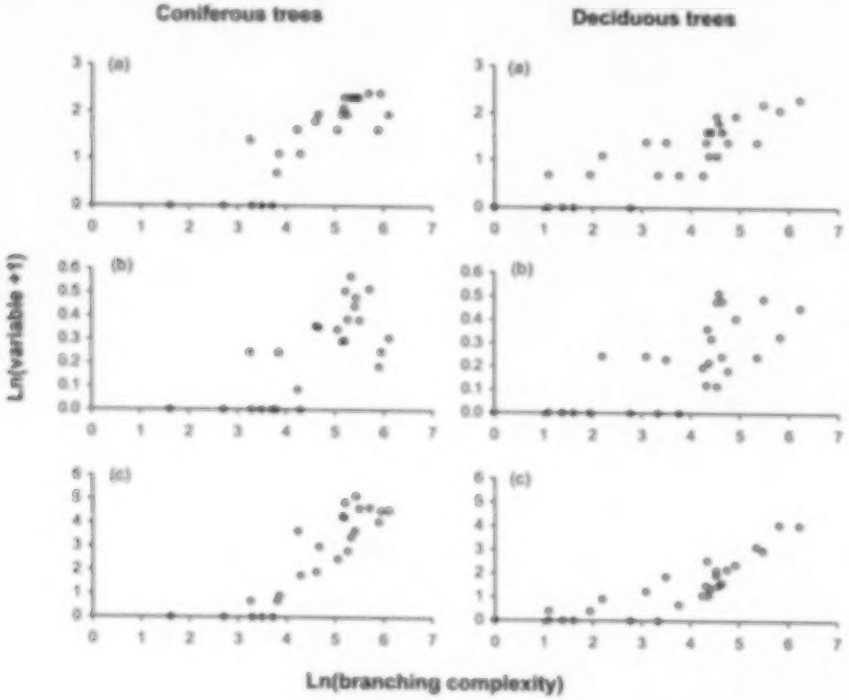
Fig. 4. Relationships between branching complexity of coniferous and deciduous trees and (*a*) taxon richness, (*b*) diversity (Shannon's index), and (*c*) total adult fish abundance in Katherine Lake. Fish species included in these analyses were muskellunge, burbot, cyprinids (*P. notatus*, *N. heterolepis*), mottled sculpin, walleye, yellow perch, johnny darter, Iowa darter, largemouth bass, smallmouth bass, black crappie, rock bass, pumpkinseed, and bluegill. Diversity and total adult abundance analyses did not include cyprinids. Ln, natural-log-transformed.



Discussion

This study demonstrates the importance of CWS as a component of littoral-zone habitat complexity, which is determined not only by the physical characteristics of CWS but by other site attributes as well (McMahon and Hartman 1989; Martin-Smith 1993; Bean and Winfield 1995). Structural habitat complexity includes CWS morphology, macrophytic structure, geomorphological characteristics (e.g., site slope, substrates, etc.), and hydrological characteristics (e.g., gradients of water clarity) utilized by fish assemblages in north temperate lakes. Complex habitats are utilized by fishes for various reasons, including as a forage substrate for insectivorous fish, as cover for refuge from predation (MacRae and Jackson 2001), as a foraging location for piscivorous fish, and as camouflage for ambush predators (Eklöv 1997), which suggests that the presence of CWS is an important characteristic of habitat complexity. Of the numerous features that could have been quantified to describe the ecological relationships between fish and habitat complexity, we focused on the morphology of CWS. In our study, characteristics that describe CWS morphology were found to be more important in describing fish distributions than were general site characteristics. Sites with CWS had greater structural diversity than sites without CWS, and structural diversity increased with branch complexity.

CWS with complex branching may act as a piscivorous foraging area, perhaps functioning in a similar manner to macrophytes. Macrophytes were not included in this study because they were absent from most of our study sites and were therefore excluded from our analyses. Macrophyte patches can have stem densities much greater than tree branching densities, but even at low or intermediate stem densities they may provide habitat similar in ecological function to CWS with complex branching. Crowder and Cooper (1982) found that bluegill density increased as macrophyte stem density increased from low to intermediate because of increased foraging efficiency at intermediate stem densities. Intermediate macrophyte stem densities may be similar to the density of the structure of complex branching. We noted that piscivorous fish in Katherine Lake, such as smallmouth bass, large yellow perch, and walleye, were more likely to be found in or around complex trees, which also have greater prey abundances. Eklöv (1997) also reported that piscivorous northern pike selected heterogeneous habitats (e.g., trees close to vegetation) to increase encounter frequency with prey, which sought cover in complex environments. Savino and Stein (1989) noted that largemouth bass and northern pike preferred heavily vegetated sites because of high prey abundance and thus increased foraging success.

Conversely, both macrophytes and wood with high levels of complex branching provide a physical barrier to large predators in search of small prey. Everett and Ruiz (1993) showed in aquarium studies that shrimp (*Palaemonetes pugio*) used sites with coarse woody debris as a refuge from predation. Manatunge et al. (2000) found that foraging effi-

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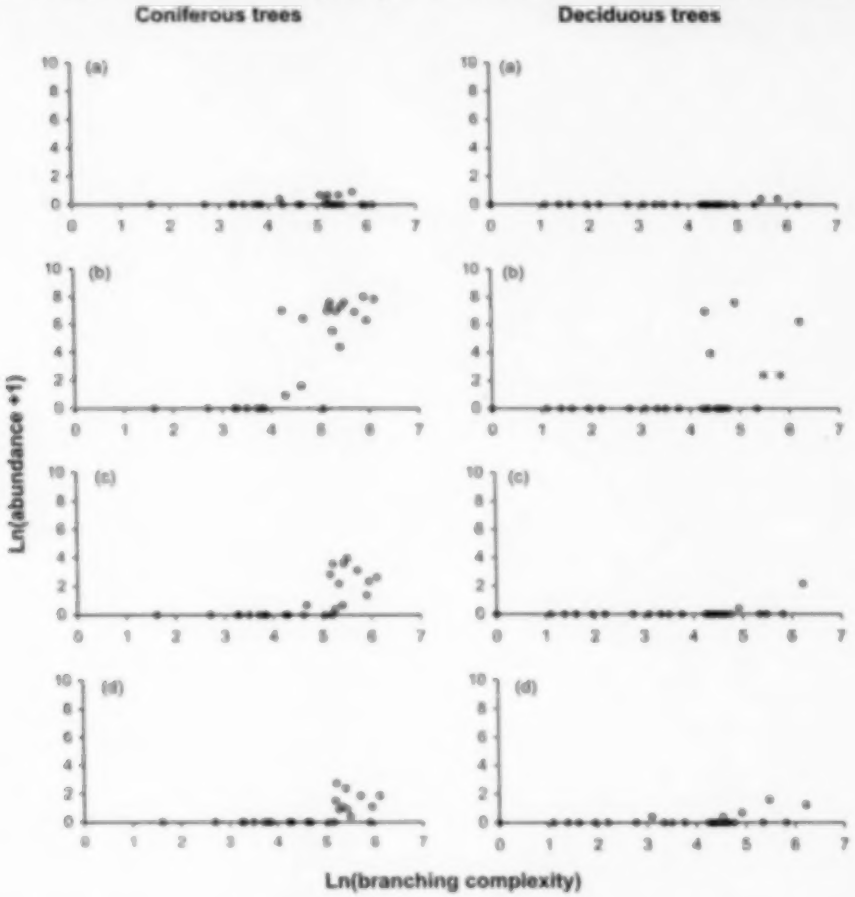
Fig. 5. Relationships between branching complexity of coniferous and deciduous trees and the abundance of (*a*) muskellunge, (*b*) cyprinids (*P. notatus*, *N. heterolepis*), (*c*) walleye, and (*d*) yellow perch in Katherine Lake. Ln, natural-log-transformed.



ciency of stone moroko (*Pseudorasbora parva*) decreased significantly with increasing habitat complexity because of impeded movement and obstructed sight. In our study, the size range of prey, including small rock bass, bluegill, and walleye, was greater in CWS with greater branching complexity. Eklöv (1997) and MacRae and Jackson (2001) found that small northern pike and other small fish inhabited dense vegetation to avoid predation. However, in our study we speculate that an asymptote of bluegill abundance may be reached at branching-complexity values between 500 and 1000, because Butler (1988) reported that bluegill abundance did not increase when plant densities increased from medium to high. In addition, Flynn and Ritz (1999) showed there was no increase in prey-capture success when stem densities increased from medium to high.

Trees with complex branching function, in part, as a foraging area or substrate for fish, thereby increasing taxon richness, diversity, and abundance of fish. Fine woody structure with greater branching complexity has more surface area and surface complexity for insect colonization, as can be found in rocky habitats (i.e., rocks with more surface complexity have greater insect densities) (Schmude et al. 1998). Woody structure also provides habitat for benthic insects (McLachlan 1987), thereby acting as a foraging substrate for insectivorous fish in Maine (Moring et al. 1986). In Katherine Lake, rock bass, bluegill, small yellow perch, small walleye, and cyprinids, which are all insectivorous species, were more abundant in complex trees.

It is clear that CWS with complex branching is important; however, there appears to be a minimum threshold of complexity at which fishes begin to congregate in trees. Taxon richness, diversity, and the abundance of most species consistently increased once branching complexity in trees exceeded 45. For yellow perch and walleye, however, a branching-complexity threshold occurred at 170 in conifers. Clearly, these thresholds have ecological implications not only for understanding the levels at which CWS becomes preferred as fish habitat, but also for the importance of tree recruitment and decomposition/erosion rates in littoral zones.

The differences between whorled and dichotomous structure provide different qualities to camouflage and cover for fish. Whorled and dichotomous branching patterns were quantified differently using the same complexity equation, and thus are not numerically comparable. The two branching patterns therefore constitute different physical and visual obstructions. Whorled branches may attract more fish because

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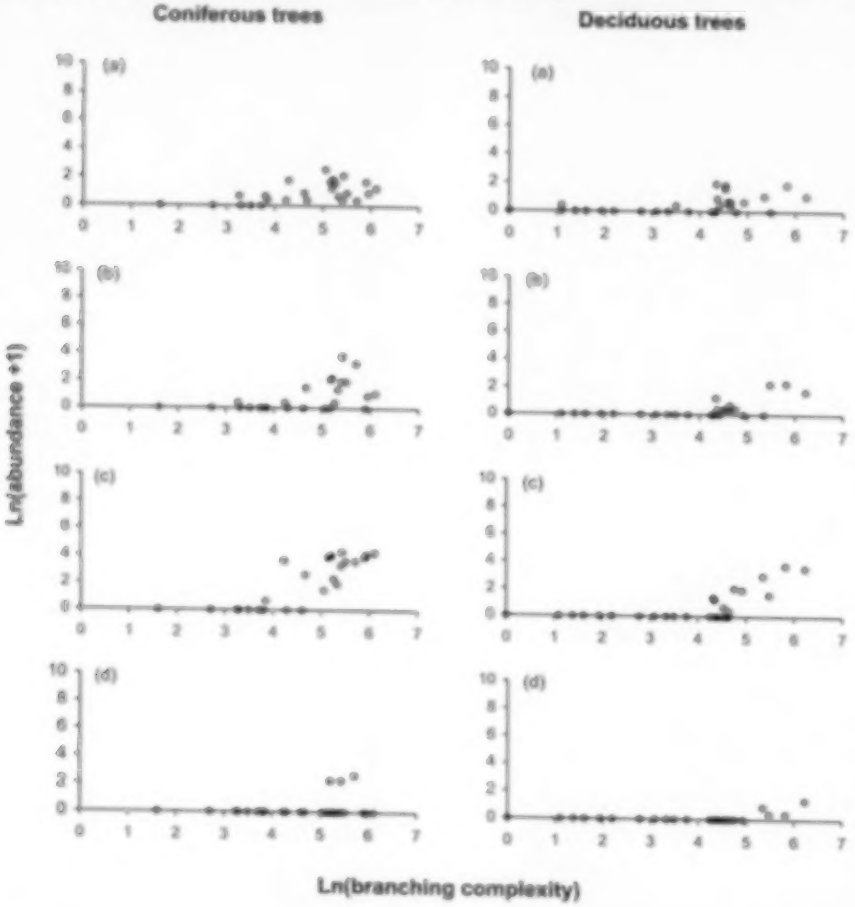
Fig. 6. Relationships between branching complexity of coniferous and deciduous trees and the abundance of (*a*) smallmouth bass, (*b*) rock bass, (*c*) bluegill, and (*d*) black crappie in Katherine Lake. Ln, natural-log-transformed.



the greater density and dispersion of branching present a barrier from a greater number of directions. The vertical and horizontal patterns in shadows probably serve as camouflage in conjunction with the barred patterns on the percids, centrarchids, and esocids. In addition, overhead shadows created by branching decrease the chances of being highlighted by sunlight.

Natural processes and anthropogenic alterations to littoral zones and riparian areas can influence CWS recruitment, branching complexity, and rates of wave and ice erosion of branches. Harvest of trees from the riparian area reduces recruitment of trees to the littoral zone (Christensen et al. 1996; Guyette and Cole 1999), thus influencing the abundance of submerged trees with complex branching. Furthermore, fine branching on newly recruited trees is naturally eliminated by erosion and decomposition. In our littoral-zone study, the greatest conifer branching-complexity value was 451 for a white pine 29 m in length. In comparison, a randomly selected standing 24.8 m tall riparian white pine with needles had a branching-complexity value of 1094. The 29 m tall littoral-zone white pine would have likely had greater branching complexity than the shorter, living white pine if it had not been exposed to wave and ice erosion. The effects of erosion and decomposition are also evidenced in Guyette and Cole's (1999) study. They found disarticulated bole and upper sections of CWS distributed in the littoral zone in the following proportions: about 44% were upper sections of the tree and 56% were boles without branches. The presence and proportions of disarticulated tree components suggest that decay and erosion reduce branching complexity such that a tree will become simpler in its geometric configuration over time.

Recreation activities can affect the rates of erosion of fine branches, thus reducing the branching complexity of trees. On numerous occasions during diving, as high-speed boats passed by and their waves contacted CWS, small submerged branches produced snapping noises. Liddle and Scorgie (1980) noted that the force of waves produced by a boat could affect macrophyte morphology and stem tensile strength, as appears to be the case for CWS. Anglers also remove branches during ice fishing for fires or to reduce over-water snags that interfere with angling. Further study is needed to identify anthropogenic effects such as erosion of branches of submerged trees and to determine the rate at which conifer-

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Table 6. Fish total length (TL) as a function of morphological and site characteristics of coniferous and deciduous trees.

Variable	Coefficient	$P_{variable}$	Intercept	n	n_{fish}	SEE	r^2	P_{model}
Coniferous								
Yellow perch TL								
Site slope	1.717	<0.001	1.314	10	74	0.413	0.308	**<0.001**
Total depth at tree	–0.099	0.015						
Smallmouth bass TL								
Mean depth at site	0.990	<0.001	0.167	20	79	0.400	0.614	**<0.001**
Freeboard (ln+1)	0.979	<0.001						
Distance to other CWS (ln(x + 1))	0.510	<0.001						
Rock bass TL								
Dominant substrate (ln)	0.359	0.015	6.594	13	159	0.544	0.226	**<0.001**
Bridging distance	0.094	<0.001						
Mean depth at site	–1.116	0.001						
Site slope	–2.893	<0.001						
Bluegill TL								
Dominant substrate (ln)	0.511	<0.001	2.560	16	722	0.558	0.274	**<0.001**
Distance to other CWS (ln(x + 1))	0.235	0.002						
Subdominant substrate (ln)	–0.216	<0.001						
Mean depth at site	–0.473	<0.001						
Freeboard (ln)	–0.689	<0.001						
Deciduous								
Yellow perch TL								
No relation	—	—	—	5	14	—	—	—
Smallmouth bass TL								
Distance to other CWS (ln(x + 1))	0.741	<0.001	1.725	14	51	0.437	0.469	**<0.001**
Freeboard (ln(x + 1))	0.392	0.025						
Bridging distance	0.309	<0.001						
Minimum depth at tree	–1.414	<0.001						
Rock bass TL								
Distance to other CWS (ln(x + 1))	1.187	0.013	2.419	10	60	0.717	0.138	**0.005**
Total depth at tree	–0.211	0.011						
Bluegill TL								
Site slope	2.770	<0.001	–3.520	10	272	0.589	0.346	**<0.001**
Mean depth at site	1.058	0.028						
Freeboard (ln(x + 1))	0.372	0.014						
Total depth at tree	0.339	<0.001						
Dominant substrate (ln)	–0.624	<0.001						

Note: Muskellunge, walleye, and black crappie were not included because no relationships could be constructed. All fish TL values were natural-log-transformed (ln); CWS, coarse woody structure; the α value was set at 0.05 for selection of each variable ($P_{variable}$); n, sample size; n_{fish}, number of fish in the analysis; SEE, standard error of the estimate; r^2, adjusted r^2; the α value for each model (P_{model}) was set at $P \leq 0.013$ using a Holm simultaneous testing procedure; Values in boldface type denote significant relationships.

ous (softwood) and deciduous (hardwood) trees lose branching complexity in relation to the rate at which they are recruited from riparian areas.

The importance of tree morphology to fish is underscored by the fact that proximity of trees to other trees increases the abundance of fish. The proximity of CWS likely plays a role in habitat partitioning in terms of risk of predation and competitive interactions. The proximity of other CWS was primarily important in describing fish TL and taxon richness. The distance to other CWS was positively related to the TL of smallmouth bass, bluegill, and rock bass, along with taxon richness. Fewer species and larger, older fish tended to inhabit isolated CWS, perhaps because the latter were less susceptible to predation while utilizing food resources. MacRae and Jackson (2001) reported that larger individuals of the same species are less prone to predation and can capitalize on other available resources in areas where the risk of predation on small individuals is high. Moyle and Vondracek (1985) found that fish were ecologically segregated (e.g., in microhabitat and diet) by age (i.e., size) to minimize competitive interactions.

In addition to branching complexity, other characteristics of CWS were also correlated with fish abundance and length and likely influenced behavioral patterns. Freeboard was important in describing fish diversity and TL and had a negative influence on fish abundance. Fish TL was also significantly related to the space above CWS. Larger piscivores, like smallmouth bass, may benefit from greater freeboard and perhaps clearance around CWS because they can forage more effectively. Eklöv (1997) documented that less dense vegetation was important to predators for foraging success. Bridging distance positively influenced the TL of fish, and

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as bridging distanced increased so did the amount of shade available for larger fish. Clearance and wet length were also important in describing fish habitat. Not surprisingly, both variables were highly correlated with branching complexity. Branches elevate boles above the substrate, thus producing more clearance, which is likely similar to freeboard in its ecological effects on fish. During this study, walleye, black crappie, bluegill, rock bass, smallmouth bass, and muskellunge were observed in the clearance and shade of the bole.

Site features associated with trees also affected the distribution of fish. Water depth was important for fish abundance and fish TL. Generally, as water depth increased so did abundance and TL of some species. Because fish tend to prefer a variety of site characteristics (Eklöv 1997), CWS habitat that encompasses a greater range of water depths may provide habitat with a greater variety of site attributes that attract fish of a greater range of TLs. Trees that were found only in shallow water provided habitats with less variability than trees spanning both shallow and deep water. For example, substrate particle sizes can decrease from shallow to deep water depending on wave base and fetch, and light can more easily penetrate to the substrate in shallow water. In our study, bluegill TL was shown to be negatively related to mean depth at conifers and positively related to mean depth at deciduous trees. We believe that conifers, which generally had greater levels of branching complexity than deciduous trees, better protected small bluegill at deeper sites than deciduous trees with relatively less complex branching at shallow sites.

Our data suggest that trees with greater branching complexity create diverse habitat. Habitat complexity at the site level encompasses depth, site slope, substrate, macrophytes, etc., and different fishes presumably respond to different physical and biological phenomena. Sites with and without wood and sites with simple and complex trees create a mosaic of habitats in the littoral zone. Lakes with and without CWS are also important in creating a mosaic of habitats. The natural characteristics of lakes, whether they have water-column structure or not, are no less ecologically important from each other. Those fish communities associated with lakes without CWS may respond to different structural characteristics, if any at all.

Our branching-complexity calculation provides a method of quantification and an index of habitat unlike that used in other studies. However, it could also have important application in other areas of research. We feel that it could be used to quantify the morphology of macrophytes for other fish habitat studies or to determine terrestrial tree branching complexity for nesting birds. Furthermore, the index of branching complexity provides a simple and fast (approximately 20 min) method of deriving the amount of branching on submerged trees with full crowns. Prior to employing the methods described in this study, our initial attempts to quantify all branches on a tree involved methods that were extremely time-consuming, taking approximately 8 h.

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Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*

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Abstract: Adult sockeye salmon (*Oncorhynchus nerka*) acquire infections with the myxosporean kidney parasite *Parvicapsula minibicornis* during their spawning migration in the Fraser River, British Columbia. Controlled infections with this parasite in wild sockeye salmon had no significant impact on plasma ionic status, metabolic rates, and initial maximum prolonged swimming performance (U_{crit}) for fish ranked as either strongly, weakly, or noninfected by polymerase chain reaction analysis of kidney tissue. However, strongly infected fish had significantly lower second U_{crit} and recovery ratio (8%) values, indicating decreased ability to recover from exercise. As the present study shows that the severity of infection is affected by time and temperature, the accumulated thermal units (ATU) of exposure in this study were compared with those experienced by naturally migrating sockeye salmon. A parallel telemetry study revealed that early-timed sockeye experienced significantly more ATU (741.4 ± 29.4 °C) than normally migrating salmon (436.0 ± 20.0 °C) prior to spawning because of a significantly longer holding period in the lake system. The present data are discussed in the context of a threshold of >450 °C ATU for severe infection that would first manifest in early-timed fish in the upper reaches of the Fraser River and certainly on the spawning grounds.

Résumé : Les saumons rouges (*Oncorhynchus nerka*) attrapent des infections à *Parvicapsula minibicornis*, une myxosporidie parasite du rein, durant leur migration de fraye dans le Fraser, Colombie-Britannique. Des infections contrôlées de ce parasite chez les saumons rouges sauvages restent sans effet sur le statut ionique du plasma, les taux métaboliques et la performance initiale maximale de nage soutenue (U_{crit}) chez des poissons classés comme fortement, légèrement ou aucunement infectés par une analyse de la réaction en chaîne de la polymérase de leurs tissus rénaux. Cependant, les poissons fortement infectés ont un U_{crit} secondaire et des valeurs du rapport de récupération (8 %) significativement plus faibles, ce qui indique une capacité réduite à récupérer après l'exercice. Puisque notre étude montre que la sévérité de l'infection est affectée par le temps et la température, nous avons comparé les unités

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thermiques cumulées (ATU) de l'exposition durant notre étude à celles subies par des saumons durant leur migration naturelle. Une étude de télémétrie faite en parallèle montre que les saumons précoces accumulent significativement plus de ATU (741,4 ± 29,4 °C) que les saumons à migration normale (436,0 ± 20,0 °C) avant la fraye, à cause d'une période significativement plus longue de rétention dans le système de lacs. Nous discutons de nos données dans le contexte d'un seuil de > 450 °C ATU pour une infection sévère qui se manifesterait d'abord chez les poissons précoces dans le cours supérieur du Fraser et certainement sur les sites de fraye.

[Traduit par la Rédaction]

Introduction

Most stocks of sockeye salmon (*Oncorhynchus nerka*) that spawn in the Fraser River watershed in British Columbia, Canada, enter the river within a week of arriving at the mouth of the estuary. In contrast, late-run sockeye salmon stocks, named for their late-summer arrival, normally congregate in the ocean near the mouth of the Fraser River for 3–6 weeks prior to initiating their upriver migration to natal spawning grounds. However, this historical migratory behaviour began to deviate in 1995 when a large portion of late-run stocks congregated for a shorter period of time and initiated upriver migration earlier than normal. Most of these early-timed fish died before reaching spawning grounds; in some years, mortality exceeded 90% (Cooke et al. 2004). Early-timed fish that survived the migrations attempted to spawn at their historically normal dates, which meant that their freshwater residence time was increased by 3–6 weeks (Cooke et al. 2004). Thus, increased freshwater residence time was associated with increased mortality prior to spawning.

Adult sockeye salmon entering the Fraser River develop detectable infections with *Parvicapsula minibicornis* (Myxozoa, Myxosporea), a microscopic waterborne kidney parasite endemic to the lowermost reaches of the river (Pacific Salmon Commission 2001; Jones et al. 2003). Outmigrating smolts also are exposed to the parasite, but it is unknown whether it is pathogenic during their marine phase or if it causes latent infection when fish mature and return to fresh water (St-Hilaire et al. 2002; Jones et al. 2003). Trophozoites of *P. minibicornis* aggregate within the kidney glomeruli (Kent et al. 1997; Raverty et al. 2000). As the infection progresses, the number of parasitic stages observed in the glomeruli increases. With severe infections, parasite development occurs in the lumen of the renal tubules, causing glomerulonephritis that leads to renal failure and mortality (Raverty et al. 2000). The infection progresses with time after first entry into the Fraser River such that it is initially only weakly detectable in kidney sections, using polymerase chain reaction (PCR) methods, in fish that have recently entered fresh water. With increased migration time, the infection is detected more intensely by PCR and eventually is detectable using histological examination of kidney glomeruli (Jones et al. 2003). Thus, the increase in freshwater residence time of early-return late-run sockeye may allow *P. minibicornis* to complete sporulation and cause damage to the glomerular capillaries and tubular epithelium sooner before spawning than for normal-timed migrants. During normal migration behaviour, maturation of the parasite coincides closely with the time of spawning (Jones et al. 2003). This led us to hypothesize that *P. minibicornis* infection may contribute directly to the accelerated mortality in early-timed fish. A loss of kidney function associated with severe *P. minibicornis* infection would be problematic for fish in fresh water because of the resulting osmotic imbalance. This loss of function would be exacerbated in migrating adult salmon because increased water uptake across the gills during swimming (Wood and Randall 1973; Gonzalez and McDonald 1994; Gallaugher et al. 2001) places additional loads on kidney function. The level of *P. minibicornis* infection that will adversely impact the physiology of sockeye salmon is unknown, but the prediction is that the intensity of kidney infection will be correlated with impaired osmoregulatory ability and swimming ability. Neither prediction has been tested experimentally, but sublethal infections of fish with other parasites such as haemoflagellates (Woo 2003), sea lice (Wagner et al. 2003, 2004), and trematodes (Coleman 1993) have been shown to negatively impact host physiology during exercise.

In the present study, we exposed wild sockeye salmon to sublethal infections of *P. minibicornis* and examined their plasma ionic status, metabolic rates, and swimming ability (maximum prolonged swimming performance, U_{crit}). By swimming fish twice with only a short recovery period (a repeated U_{crit} test; Jain et al. 1998), the expectation was that any physiological disturbance accrued in the first swim would be exacerbated in the second one and hamper performance (Tierney and Farrell 2004). Ionic and osmotic status was measured from blood samples collected after the second swim. Data from a parallel telemetry study were used to compare the estimated parasite impact on migration ability with actual migrations of early-timed and normally behaving sockeye.

Materials and methods

Fish capture and holding

The geographic locations of all sockeye capture and experimental sites for the present study are provided in Fig. 1. The fish used in experimental trials were part of a larger group of approximately 240 wild sockeye salmon captured by a commercial seine boat in the Strait of Georgia near Vancouver, British Columbia, on 26 and 28 August 2003. All fish were landed at the Fisheries and Oceans Canada West Vancouver Laboratory. To avoid exposure to the main stem of the Fraser River and hence prevent adult salmon from encountering *P. minibicornis*, we immediately transferred a group of fish that we termed "control, unexposed" (mean ± SE: 61.0 ± 0.9 cm, 2.57 ± 0.16 kg, $n = 10$) to the Fisheries and Oceans Canada Cultus Lake Laboratory and

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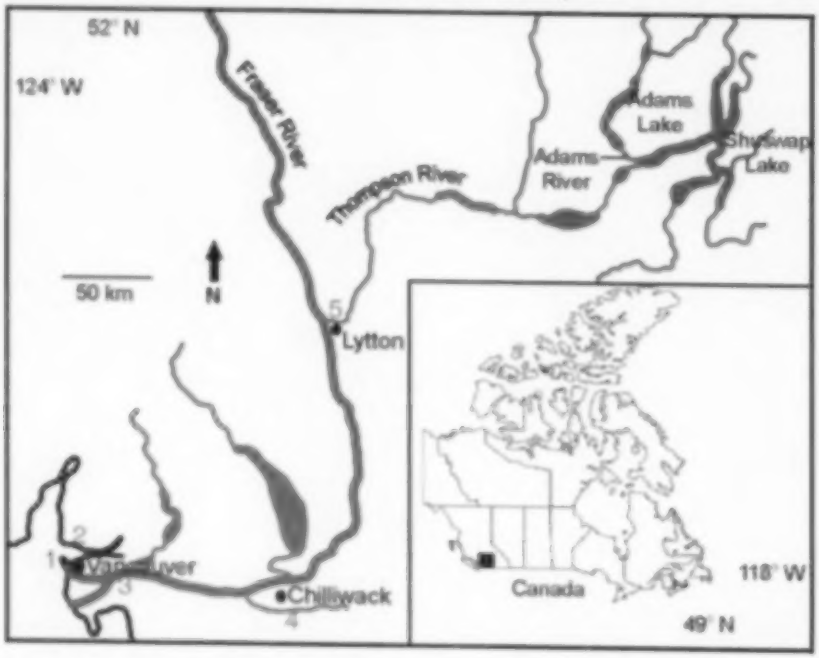
Fig. 1. Map of the lower Fraser River, Thompson River, and Shuswap Lake areas in British Columbia indicating the location of ocean capture (1), the West Vancouver Laboratory (2), the Annacis Island facility (3), the Cultus Lake Laboratory (4), and the Thompson River capture site (5). The solid region in the inset map of Canada indicates the study area.



placed in 20 000-L holding tanks with a flow-through supply of fresh water (12.7 ± 0.3 °C) obtained directly from Cultus Lake. To provide a controlled exposure to lower Fraser River water, we transported a group of fish (60.7 ± 1.0 cm, 2.63 ± 0.18 kg, $n = 10$) from the West Vancouver Laboratory to Fisheries and Oceans Canada Annacis Island and placed in two 7000-L holding tanks where they became naturally infected with *P. minibicornis* over a 7-day period through the flow-through supply of water directly from the Fraser River (15.6 ± 0.1 °C). Water at this location has been shown to carry infectious stages of the parasite (Jones et al. 2003). Infected fish subsequently were transferred to the Cultus Lake Laboratory and kept in conditions similar to those of control fish. The number of accumulated thermal units (ATU) (daily cumulative temperature above 0 °C commencing from initial exposure to Fraser River water) was calculated because progress of the myxozoan life cycle is positively correlated with temperature and incubation time (Hendrickson et al. 1989; McGeorge et al. 1996; Redondo et al. 2002). We collected an additional 10 adult late-run sockeye (60.1 ± 1.2 cm, 2.61 ± 0.15 kg) from the Thompson River canyon on 20 September 2003 and transported them to the West Vancouver Laboratory where they were kept in a 7000-L holding tank with a flow-through supply of fresh water (12.6 ± 0.2 °C). These fish had completed 300 km of their 500-km spawning migration. Late-run sockeye salmon carrying transmitters were tracked by radiotelemetry from the Fraser River mouth using fixed stations until they entered Shuswap Lake, adjacent to the spawning grounds (English et al. 2004). Using this information, we estimate these fish had been migrating upriver for 13 days and thus were exposed to approximately 203 °C ATU. Four of these fish were lost prior to testing from unidentified causes.

Routine blood samples

Blood was sampled from a subgroup of cannulated infected and uninfected fish to assess ionic status (plasma chloride, potassium, sodium glucose, lactate, and cortisol levels). Sockeye salmon were individually netted and anaesthetized in a 60 mg tricaine methanesulfonate·L^{-1} (MS 222) bath and placed on a surgery table with a flow-through maintenance bath of 30 mg MS 222·L^{-1}. A dorsal aorta cannula was inserted using an internal trochar, anchored in place using 3-0 silk suture, and filled with 0.9% heperanized saline. Fish were then placed into one of four 80-L isolation chambers with a water flow rate of 10 L·min^{-1} to recover for 14–20 h. Four 1-mL blood samples were taken over a 2-day period. Each blood sample was centrifuged and the plasma stored at –80 °C until the analyses were performed (as described in Farrell et al. 2001). Samples of gill filaments were frozen for enzymatic analysis of Na^+/K^+-ATPase activity performed according to McCormick (1993). Opercular tissue DNA was analysed according to Beacham et al. (2004) to confirm that fish were from the Adams River stock. As well, somatic energy levels were measured on each fish using a Distell fat meter (Distell.com, Old Levenseat, Fauldhouse, West Lothien, Scotland, UK) following the methods of Crossin and Hinch (2005).

Swimming performance

Owing to the constraint that only one fish could be tested daily, control and exposed fish were tested in split batches

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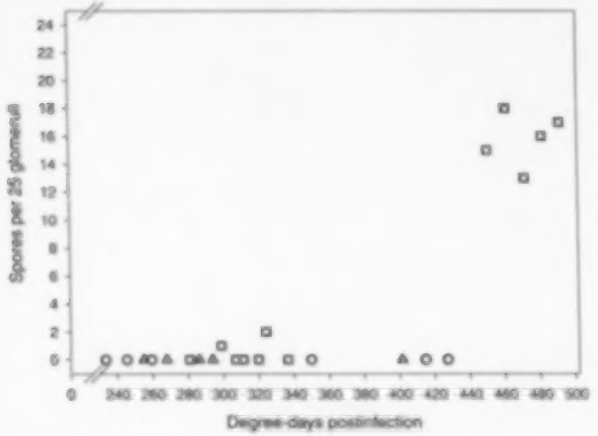
Fig. 2. Infection levels of sockeye salmon (*Oncorhynchus nerka*) exposed to *Parvicapsula minibicornis* in the Fraser River for varying accumulated thermal units (ATUs) (cumulative daily water temperature above 0 °C in four locations along the migration route). Infection severity was estimated histologically from the mean number of infected glomeruli per 25 glomeruli (*y* axis) and by polymerase chain reaction amplification of 1091 base pair fragments of the *P. minibicornis* 18S rRNA gene present in kidney tissue (circles, uninfected; triangles, weak infection; squares, strong infection). All uninfected fish are controls not exposed to the parasite.



consisting of five salmon over an 8-week period that resulted in a graded level of infection among the infected group (see Fig. 2). Fish were netted individually and anaesthetized in a 60 mg MS 222·L^{-1} bath for approximately 5 min prior to taking body measurements and transferred to the swim tunnel (471-L Brett-type as described in Farrell et al. (2003) with a modified net plug to prevent drafting in the turbulent area near the upstream end). Fish were introduced into the swim tunnel in early afternoon and allowed to acclimate for 2 h at a water velocity of 0.45 body length·s^{-1} prior to performing a conditioning U_{crit} test (Jain et al. 1997). For this test, water velocity was increased by 0.15 body length·s^{-1} every 2 min until the fish could no longer swim against the current and rested for more than 20 s on the downstream grid. Fish were allowed to recover overnight (14–16 h) at 0.45 body length·s^{-1}. The next morning, routine oxygen uptake ($M_{O_2,routine}$) was estimated from two 15-min recordings taken twice during a 1-h period. The lowest value was used for $M_{O_2,routine}$, although the two values rarely differed by more than 5%. To measure oxygen uptake, we pumped swim tunnel water past an oxygen meter (Mark IV Oxyguard; Point Four Systems, Richmond, British Columbia) connected to a computer that logged oxygen measurements every 5 s using Labview software. A ramped U_{crit} protocol (Jain et al. 1997, 1998) was initiated by increasing water velocity 0.15 body length·s^{-1} every 5 min to ~50% of initial U_{crit} and subsequently every 20 min until fish fatigued. The U_{crit} values were calculated using the formula of Brett (1964) and corrected for solid blocking effects when necessary (10%–20% difference) according to Bell and Terhune (1970). Active M_{O_2} values were measured for every velocity increment of the U_{crit} test. Fish were permitted to recover for 45 min at 0.45 body length·s^{-1}, and $M_{O_2,routine}$ was remeasured for 15 min immediately prior to a second ramped U_{crit} test to examine the ability of fish to recover from the initial exhaustive exercise. Recovery ratios for U_{crit} and $M_{O_2,max}$ were calculated by dividing the results of the two swim tests ($U_{crit2} \cdot U_{crit1}^{-1}$ and $M_{O_2max2} \cdot M_{O_2max1}^{-1}$, respectively) for each fish. The recovery ratios of wild sockeye salmon typically equal unity (i.e., recovery ratios = 1.00) (Farrell et al. 2003; Lee et al. 2003). Therefore, if a recovery ratio was significantly <1.00, fish were considered to have failed to fully recover from the initial swim trial. The M_{O_2} recordings were continued for 30 min after the end of U_{crit2} to determine rates of recovery from exhaustive exercise. At the end of the second recovery period, fish were removed immediately from the swimming chamber and euthanized in 140 mg MS 222·L^{-1}. Blood was sampled by caudal puncture for analyses of the plasma. Plasma and all other tissues were analysed similar to cannulated fish.

Assessments of infection prevalence and severity

Duplicate posterior kidney samples were fixed and analysed for the presence of *P. minibicornis* by histology and PCR methods according to Jones et al. (2003). For histological analysis, duplicate 5-µm sections of kidney fixed in Davidson's solution were mounted on glass slides and stained with haematoxylin and eosin. Histological samples were scored infected when myxozoan trophozoites were observed in glomeruli, and the severity of infection was estimated from the mean number of infected glomeruli per 25 glomeruli examined under a light microscope. A severe level of infection was >10 spores per 25 glomeruli (Jones et al. 2003). For PCR analysis, kidney samples fixed in 95% ethanol had DNA extracted and amplified using the same primers and conditions described by St-Hilaire et al. (2002). Samples were scored positive if a 1091 base pair fragment of the *P. minibicornis* 18S rRNA gene was detected. Results for the PCR analysis were classified into three categories by an individual technician: uninfected (no PCR product amplified), weak (barely detectable 1091-bp product amplified), and strong (an intense 1091-bp product amplified). Levels of infection severity are compared as a function of ATU for both assessment methods (Fig. 2).

Modelling thermal exposure

River migration travel rates for 51 sockeye salmon from the Adams River late-run stock were obtained from a multistock radiotelemetry study conducted on Fraser River sockeye in 2003 (English et al. 2004). In their study, sockeye were captured using a commercial seine boat in Johnstone Strait near Campbell River, British Columbia (50.12°N, 125.21°W). These fish were gastrically implanted with radiotransmitters (16.1 g, 16 mm × 51 mm, MCFT-3A; Lotek Wireless Inc., Newmarket, Ontario) and tracked using SRX_400 receiver stations located in the lower Fraser River (Mission, British Columbia, 49.08°N, 122.18°W, 10 m above sea level), at the entrance to the holding lake adjacent to the main spawning stream (Shuswap Lake, 50.42°N, 119.16°W, 345 m above sea level), and at the entrance to the

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spawning grounds (Adams River, 50.90°N, 119.55°W, 345–360 m above sea level). Water temperatures encountered by migrating sockeye were calculated from temperature stations located just upstream from the mouth of the Fraser River (Whonnock, British Columbia, 49.17°N, 122.46°W), at the midpoint of their migration in the Fraser River (Hope, British Columbia, 49.38°N, 121.43°W), in the Thompson River near its confluence with the Fraser River (Lytton, British Columbia, 50.23°N, 121.58°W), and at completion of river migration at the entrance to Shuswap Lake (Chase, British Columbia, 50.82°N, 119.68°W) at the entrance to Shuswap Lake where the sockeye hold prior to entering their spawning grounds in the Adams River. As the exact location of sockeye migrating through Shuswap Lake is unknown, we used both a maximum exposure temperature (assuming fish that held in the epilimnion, which was estimated from the temperature of water flowing from the lake entrance) and a minimum exposure temperature (assuming that fish held in the hypolimnion, which is normally stable at 8 °C from September through November within a few metres of the bottom (Nidle and Shortreed 1996)). Migrants were classified as early-timed if they entered the Fraser River 13 days or fewer after initial capture and tagging in Johnstone Strait (English et al. 2004).

Statistical analyses

Values presented are means ± SE and statistical significance was assessed at $P < 0.05$ using a standard statistical program (JMP 4.0.4; SAS Institute Inc., Cary, North Carolina). Histological, PCR, and blood data were analysed by one-way ANOVA. Oxygen consumption and U_{crit} data were analysed by one-way ANOVA for comparisons between treatments and one-way repeated-measures ANOVA for comparisons between U_{crit1} and U_{crit2}. Recovery ratios were transformed for statistical analysis and compared with a value of unity using one-sample t tests followed by Bonferroni adjustments to account for multiple comparisons.

In all cases, parasite infection level was the independent variable, and fish length, mass, and water temperature were tested for covariation and included if significant. Tukey's multiple comparison method was used to determine which treatments differed if ANOVA indicated statistically significant differences. Migration data were analysed by Mann–Whitney rank sum tests because of unequal variances for migration days and ATU of early and normal-behaving sockeye.

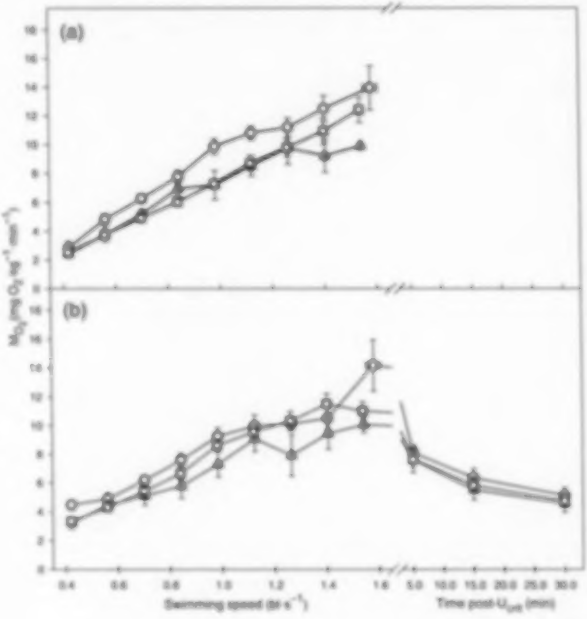
Results

Parasite infection

Histological analyses determined that spores were absent from kidney glomeruli of all control fish and all fish collected from the Thompson River. Fish exposed at Annacis Island became infected with *P. minibicornis* and those exposed and held for more than 450 °C ATU averaged 15.8 spores per 25 glomeruli. In contrast, fish tested prior to the 350 °C ATU incubation period had either very low or no spores in the glomeruli.

PCR analyses detected *P. minibicornis* infection much earlier than histological analysis. All fish exposed at Annacis Island were PCR positive for *P. minibicornis*, as were all fish captured from the Thompson River (Fig. 2). However, only a weak PCR response was observed in fish tested prior to 300 °C ATU incubation. A strong parasite presence was always indicated by PCR for all exposed fish with >300 °C ATU incubation and spores were observed in these fish. One control fish had a weak parasite presence based on PCR analysis and was therefore reassigned to the weak PCR parasite grouping for subsequent analysis. This fish was tested at 400 °C ATU and is the one fish with weak PCR presence above 300 °C ATU. The remaining control fish were PCR negative.

Fig. 3. Rates (mean ± SE) of oxygen uptake ($\dot{M}_{O_2}$) for sockeye salmon (*Oncorhynchus nerka*) with uninfected (circles) or weak (triangles) or strong (squares) kidney infections of *Parvicapsula minibicornis* (as measured by polymerase chain reaction analysis) during the (*a*) first and (*b*) second swim test. The 30-min recovery period during the second swim test is also shown. Routine oxygen uptake ($\dot{M}_{O_2\,routine}$) was taken from the slowest swimming speed (0.45 $m \cdot s^{-1}$). While $\dot{M}_{O_2}$ increased with swimming speed, values were not significantly different ($P > 0.05$) between groups for either swimming trial.



Metabolic rates and swimming performance

The $\dot{M}_{O_2}$ values increased with swimming speed in all groups, as expected (Fig. 3), but the level of parasite infection had no significant effect on either $\dot{M}_{O_2 routine}$ or $\dot{M}_{O_2 max}$ (Table 1). Similarly, active $\dot{M}_{O_2}$ during swimming and recovery did not differ significantly with infection.

While U_{crit1} and U_{crit2} values did not differ significantly between treatments, sockeye with strong PCR parasite infection levels performed significantly poorer during U_{crit2} compared with U_{crit1} (Table 1). Only strongly infected fish had recovery ratios significantly below unity (Table 1), and a comparison of the U_{crit} recovery ratios and the ATU for each

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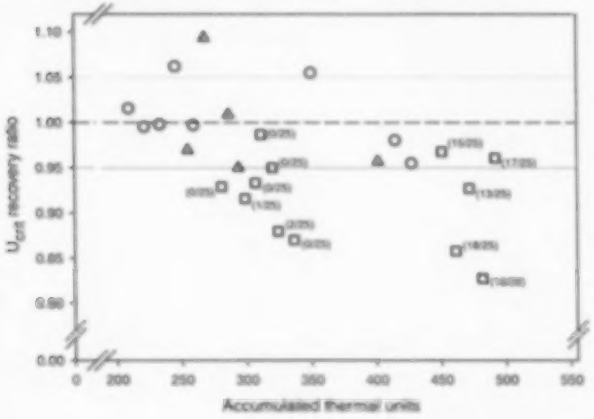
Table 1. Routine metabolic rates, maximum metabolic rates ($M_{O_2,max}$), repeated maximum prolonged swimming performance (U_{crit}), and recovery ratios ($M_{O_2,max2} \cdot M^{-1}_{O_2,max1}$ and $U_{crit2} \cdot U^{-1}_{crit1}$) for sockeye salmon (*Oncorhynchus nerka*) with different levels of *Parvicapsula minibicornis* infection (measured by polymerase chain reaction).

Parasite presence	Routine metabolic rates ($mg\ O_2 \cdot kg^{-1} \cdot min^{-1}$)	$M_{O_2,max1}$ ($mg\ O_2 \cdot kg^{-1} \cdot min^{-1}$)	$M_{O_2,max2}$ ($mg\ O_2 \cdot kg^{-1} \cdot min^{-1}$)	M_{O_2} recovery ratio	U_{crit1} (body lengths·s^{-1})	U_{crit2} (body lengths·s^{-1})	U_{crit} recovery ratio
Uninfected (n = 9)	2.83±0.50	13.94±1.54	14.18±1.78	1.02±0.04	1.56±0.07	1.57±0.07	1.01±0.01a
Weak (n = 5)	2.42±0.23	9.89±0.18	10.08±0.56	1.02±0.04	1.59±0.07	1.55±0.03	0.99±0.03a
Strong (n = 12)	2.52±0.20	12.50±1.08	11.70±0.70	0.95±0.03	1.52±0.06	1.40±0.06*	0.92±0.01b

Note: Data are means ± SE. Fish were swim tested in two separate trials with a 45-min recovery period between them. The asterisk indicates that the U_{crit2} and recovery ratio of strongly infected fish were significantly ($P < 0.001$) lower than U_{crit1}. Dissimilar letters indicate significant ($P < 0.05$) differences between treatments.

Fig. 4. Critical swimming speed recovery ratios for sockeye salmon (*Oncorhynchus nerka*) with uninfected (circles) or weak (triangles) or strong (squares) kidney infections of *Parvicapsula minibicornis* (as measured by polymerase chain reaction analysis) exposed to a given number of accumulated thermal units (ATUs) (cumulative daily water temperature above 0 °C in four locations along the migration route). Parenthetical data next to strongly infected fish indicate the number of kidney glomeruli, determined out of 25 by histological analysis, with spores present. The dashed line indicates the line of unity (i.e., performances in the first and second swim tests were identical) and the dotted lines indicate the expected resolution for recovery ratios (±5%).



fish shows that the majority of these fish fell below the expected recovery ratio resolution (±5%) (Fig. 4).

Tissue variables

Routine and postexercise plasma ion levels (chloride, potassium, and sodium) did not differ significantly with disease or with exercise (Table 2). Routine plasma glucose and cortisol levels also did not differ significantly with disease or with exercise. Plasma lactate levels increased significantly ($P < 0.01$) with exercise for the uninfected and strongly infected treatments but not for fish with weak infections. Significant differences in plasma osmolality occurred between infected fish at rest and uninfected fish after exercise. Gill Na^+/K^+-ATPase activity after exercise was significantly lower (weak, $P < 0.01$; strong, $P < 0.03$) in infected fish compared with resting uninfected fish but was unchanged by exercise in control fish. Whole-body energy levels did not differ significantly between the three treatments after exercise, thereby eliminating the possibility that infected fish were in a poor physical condition.

Modelling thermal exposure

Late-run sockeye salmon that began river migration earlier than normal required a similar number of days (24.4 ± 1.2) as normally timed fish (22.2 ± 0.7) to migrate from the mouth of the Fraser River to the Shuswap Lake holding area (Fig. 5). Early migrants also held in Shuswap Lake for a significantly longer period (20.0 ± 1.9 days) ($P < 0.001$) than normally migrating salmon (6.5 ± 0.7 days) before migrating to their spawning grounds (Fig. 5). Early timing also results in exposure to warmer temperatures and as a result, early migrants were exposed to significantly more ATU (429.7 ± 13.3 °C) ($P < 0.001$) than normal-timed fish (333.0 ± 10.1 °C) during upriver migration (Fig. 6). Thus, prior to entering the spawning grounds, early-timed fish had experienced significantly ($P < 0.001$) higher ATU (minimum = 592.4 ± 21.8 °C, maximum = 741.4 ± 29.4 °C) compared with normally migrating salmon (minimum = 384.9 ± 11.2 °C, maximum = 436.0 ± 20.0 °C) prior to entering the spawning grounds (Fig. 6). Based on the dates and migration times of fish in the telemetry study (English et al. 2004), sockeye captured from the Thompson River for the physiological study were deemed to exhibit normal migration behaviour.

Discussion

Our primary purpose was to determine if a controlled *P. minibicornis* infection affects the repeated prolonged swimming performance of adult wild sockeye salmon and, if a negative effect occurred, to assess the potential impact on sockeye migratory ability. Although a controlled experimental infection with *P. minibicornis* has never been performed previously with adult salmon, the ability to infect with lower Fraser River water is consistent with the prediction that this is the natural site of transmission of *P. minibicornis* (St-Hilaire et al. 2002; Jones et al. 2003). The life cycle of *P. minibicornis* is not fully understood but like other myxosporeans is expected to include an invertebrate alternate host that releases actinospores into the water column, in this case the lower Fraser River. The absence of detectable infections in most unexposed salmon further confirmed that salmon acquire infections following entry into the Fraser River during the spawning migration rather than in a latent fashion via

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Table 2. Routine and postexercise (taken after 30 min of recovery from U_{crit}) plasma variables, gill enzyme activity, and whole-body energy of sockeye salmon (*Oncorhynchus nerka*) with different levels of *Parvicapsula minibicornis* infection (measured by polymerase chain reaction).

	Routine*		Post-U_{crit}		
Blood variable	Uninfected (n = 8)	Strong (n = 8)	Uninfected (n = 4)	Weak (n = 5)	Strong (n = 12)
Sodium (mmol·L^{-1})	153.7±1.6	152.2±1.0	162.6±4.7	161.8±11.6	157.9±3.7
Chloride (mmol·L^{-1})	124.2±3.6	124.6±1.4	117.0±3.5	120.2±2.2	119.0±0.8
Potassium (mmol·L^{-1})	2.6±0.5	2.3±0.2	1.4±0.1	2.0±0.5	3.3±0.4
Osmolality (mosmol·kg^{-1})	288.8±4.8ab	274.0±3.4a	326.8±23.3b	301.6±10.7ab	290.8±3.0ab
Glucose (mmol·L^{-1})	6.4±0.4	6.3±0.4	7.0±0.7	7.5±0.9	7.4±0.7
Lactate (mmol·L^{-1})	2.0±0.9a	1.4±0.3a	6.1±1.2b	2.9±0.9ab	4.6±0.9b
Cortisol (ng·mL^{-1})	125.4±17.9	156.0±24.4	228.9±82.0	225.8±19.5	188.7±41.3
Na^+/K^+-ATPase (μmol ADP·mg protein^{-1}·h^{-1})	1.8±0.1a	1.3±0.2ab	1.4±0.3ab	0.7±0.2b	1.1±0.2b
Somatic energy (MJ·kg^{-1})	6.8±0.5	6.5±0.3	5.9±0.3	6.2±0.3	6.5±0.2

Note: Data are means ± SE. Dissimilar letters indicate significant differences ($P < 0.05$) between treatments.
*Samples taken from cannulated fish in 80-L isolation chambers.

Fig. 5. Mean (±1 SE) cumulative number of days taken by late-run sockeye salmon (*Oncorhynchus nerka*) that entered the Fraser River early (≤13 days after capture and tagging (hatched bars)) and those that held in the ocean prior to river entry (>13 days after capture and tagging (open bars)) to migrate from the mouth of the Fraser River to Shuswap Lake and from the Fraser River mouth to their final spawning grounds in the Adams River. The asterisk indicates that the value is significantly ($P < 0.05$) higher for early-migrating sockeye.

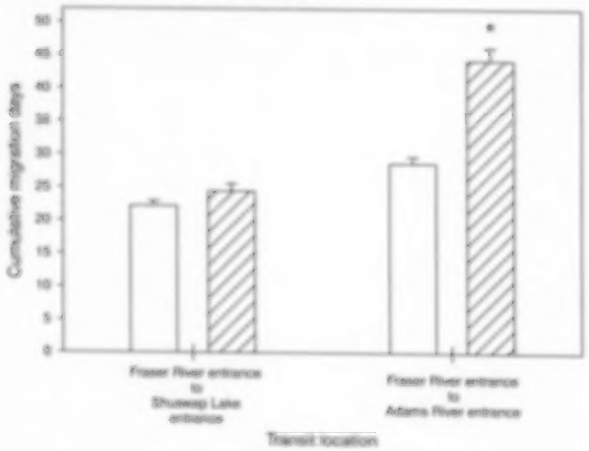
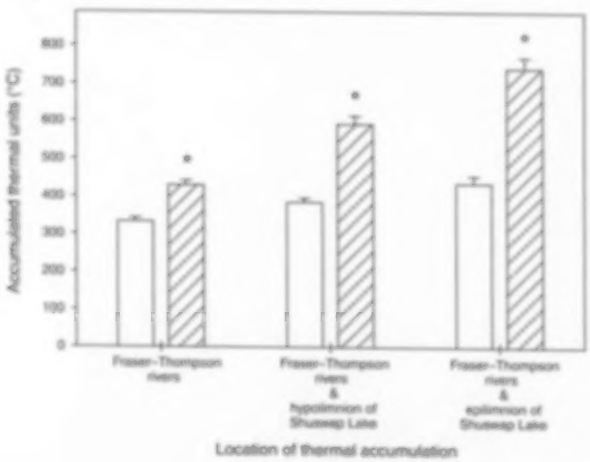


Fig. 6. Mean (±1 SE) accumulated thermal units (ATUs) (cumulative daily water temperature above 0 °C in four locations along the migration route) by early-timed (hatched bars) and normal-timed (open bars) late-run sockeye salmon (*Oncorhynchus nerka*) passing through the river segments of their migration (Fraser River mouth to Shuswap Lake) and passing through the entire migration route including river and lake segments (Fraser River mouth to the spawning ground entry). Because fish locations in Shuswap Lake are unknown and the lake thermally stratifies in the summer, minimum (hypolimnion exposure) and maximum (epilimnion exposure) ATUs are presented for the cumulative river and lake segments. Asterisks indicate significant difference ($P < 0.05$).



contact during the smolt out-migration. The infection in the single unexposed salmon may have been due to the chance exposure of this fish to actinospores that had entered the Strait of Georgia from the river or to the swimming behaviour of this fish having brought it closer to the river estuary. Previous studies failed to find evidence of infection in over 540 adult sockeye that had been collected from the west coast of Vancouver Island or the lower Fraser River prior to accruing sufficient ATU for parasite development (St-Hilaire et al. 2002; Jones et al. 2003). Also in agreement with earlier studies was the finding that PCR analysis identified *P. minibicornis* infections much earlier than histological analysis (St-Hilaire et al. 2002; Jones et al. 2003). In the present study, a weak PCR infection consistently appeared at ~150 °C ATU before the appearance of spores, an interval that translates to about 2 weeks at 12 °C. Infected sockeye that experienced >450 °C ATU had more severe PCR infections and histological infections (13–18 glomeruli infected; according to Jones et al. (2003), severe ≥10 per 25 glomeruli infected), while those with <350 °C ATU had a weak PCR infection and few spores. Therefore, the period between 350 and 450 °C ATU seems to be necessary for the proliferation of spores in the glomeruli of late-run sockeye salmon under the present conditions. By implication, any significant im-

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pact associated with infection likely will require exposure levels of >500 °C ATU to reach glomeruli infection levels nearing 25, which are commonly seen in wild fish (Jones et al. 2003). Fish in the present study did not reach the level of ATU exposure needed to produce the severe kidney damage found by Raverty et al. (2000).

Despite the severe levels of kidney infection in some fish, the initial metabolic rates at rest and during exercise were within the range of those found in previous studies on wild adult sockeye (Farrell et al. 2003; Lee et al. 2003). This lack of impairment also was true for the initial U_{crit} of fish regardless of infection severity. However, fish with strong *P. minibicornis* infections averaged a significantly lower second U_{crit} and reduced recovery ratio, indicating a decreased the ability to recover from exercise. This 8% reduction in U_{crit} recovery ratio is similar to that found by Jain et al. (1998) for sockeye salmon exposed to the toxicant dehydroabietic acid. The decrease in recovery ratio was not dependent on exposure time because the majority of strongly infected fish fell below the expected recovery ratio resolution regardless of ATU. Therefore, it appears that fish can experience a physiological impact similar to that of a toxicant before *P. minibicornis* spores reach high numbers. However, ATU exposure of fish in the present study is below that experienced in-river by wild sockeye salmon, and it is most likely that this impact will be much greater with longer ATU exposure. Given that metabolic rates were similar between treatments, a reduction in swim performance may be the first indicator that physiological problems associated with *P. minibicornis* are beginning.

Given that *P. minibicornis* causes kidneys damage, poorer recovery of severely infected fish in the absence of significant effects on metabolic rates could mean that the osmoregulatory abilities of fish are beginning to fail. Raverty et al. (2000) previously hypothesized that a relationship exists between kidney tissue damage and an impairment of renal function during severe infections, possibly leading to mortality. Osmoregulatory failure was implicated in a significant decline in swimming performance without a change in cardiac output for Atlantic salmon infected (*Salmo salar*) with sea lice (Wagner et al. 2003). Even so, the strong *P. minibicornis* infection produced here (12–18 of 25 glomeruli infected) had no significant effect on the plasma ion and osmolality levels for sockeye salmon at rest. Ion and osmotic disturbances can be delayed in their appearance, but no such disturbances were observed after exercise. Plasma lactate levels also did not differ between treatments postexercise, indicating that muscle activity likely was comparable.

The early stages of glomerulonephritis that precede eventual renal failure in severely infected fish (Kent et al. 1997; Raverty et al. 2000) could be compensated for by increased reliance on the gills for ionoregulation, thereby maintaining plasma ionic status as observed here. The activity of chloride cells in the gills normally increases to compensate for the passive ion loss and water entry in fresh water (Schreck 1990; Wang et al. 1994; Wendelaar Bonga 1997). However, an up-regulation of the Na^+/K^+-ATPase ion pump was not observed in infected fish. In fact, Na^+/K^+-ATPase activity was significantly lower in infected fish postexercise compared with uninfected resting fish but was not significantly different from that of uninfected fish postexercise. It is important to note that in the present study, postexercise tissue samples were not taken until after a 30-min recovery period (which was needed to measure excess postexercise oxygen consumption). We cannot exclude the possibility that this amount of time may have been long enough for the tissue variables to stabilize from exercise and account for the lack of any significant postexercise changes.

We are able to place the results for the present controlled infection in the context of naturally infected early- and normal-migrating late-run salmon. The radiotelemetry tracking study (English et al. 2004) clearly revealed that both normal- and early-timed late-run sockeye travelled from the mouth of the Fraser River to their holding lake in about the same amount of time. Therefore, the present results clearly show that the primary difference in incubation ATU lies in the extra time that early-timed fish hold in the lake before spawning. Early migrants also experience higher river temperatures, further increasing the difference in incubation ATU. Given the observed threshold of 450 °C ATU for fish to begin to manifest severe *P. minibicornis* infection (i.e., a strong PCR result plus histological presence of ≥10 of 25 infected glomeruli), this threshold would have been approached but not exceeded when early migrants attempted to pass through the Thompson River canyon. This stretch of river is the last major hydraulic challenge during the migration of this stock. Therefore, the present results suggest that the severity of *P. minibicornis* at this stage of the migration would not impact on repeat swimming performance. Whether the progression of the infection may influence spawning physiology in the lake and beyond, when energy still is required to hold position in the river while competing for mates and defending redds (Healey et al. 2003), cannot be determined by the present study.

We did not assess for graded effects of increasing incubation between 300 and 490 °C ATU, nor were longer incubation periods studied. Nevertheless, the present analysis suggests that such studies are needed to fully assess the physiological impact of *P. minibicornis*. Depending on where fish hold in the lake, incubation could reach minimally 600 °C ATU (i.e., holding in the hypolimnion) and maximally 750 °C ATU (i.e., holding in the epilimnion) by the time early-timed fish reach the spawning grounds in the Adams River. Obviously, fish holding in the lake hypolimnion could benefit substantially in terms of retarding parasite infection compared with fish holding in the epilimnion. In comparison, the successfully spawning normal-timed late-run sockeye are estimated to have approximately 400 °C ATU of incubation regardless of location within the lake. This period of incubation corresponds to the point where spore proliferation likely begins to become severe, although apparently not enough to negatively affect spawning ability. Furthermore, the PCR signal would likely indicate a strong infection for normal-timed spawning ground sockeye, suggesting that while PCR may be an advantageous tool for early detection of the parasite, spore appearance in the glomeruli may be more useful in predicting adverse consequences of infection likely to occur at higher ATU exposure.

Based on our findings and the observations of naturally migrating late-run sockeye salmon, we have eliminated the

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possibility that *P. minibicornis* impact the metabolic rates, repeat swimming performance, or osmotic disruption of these fish within the main stem of the Fraser and Thompson Rivers. However, we only assessed the impact for up to 490 °C ATU, and yet it is clear from the telemetry data that early-timed migrants of these stocks can experience an additional incubation period of 100–250 °C ATU. During this time, the ability of sockeye salmon to recover from exercise is likely to deteriorate and possibly affect their ability to avoid predators, traverse the spawning grounds, hold station, and compete for mates. Therefore, further studies using extended exposure intervals and a broader range of assessment endpoints are required to fully evaluate the impact of this parasite on the physiology of early-timed sockeye salmon in the Fraser River.

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Modelling ice cover, timing of spring stratification, and end-of-season mixing depth in small Precambrian Shield lakes

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Abstract: From a 3-year study of 21 northern Ontario lakes, models were developed to determine the duration of ice cover, predict the timing of spring stratification, and predict the end-of-season mixing depth. The model to determine lake freeze and thaw dates was based on the daily variability of water temperature measured with a data logger suspended 1 and 2 m below the surface. The model to predict the duration of time (days) from lake thaw to lake stratification was developed using the mean May air temperature (degrees Celsius), dissolved organic carbon, and lake surface area (r^2 = 0.79). The end-of-season mixing depth was best predicted using days to stratification and dissolved organic carbon concentration (r^2 = 0.72). By applying a simple climate change scenario model, we were able to show that increased air temperature, rather than increased water clarity, was the most important factor affecting the timing of stratification. In contrast, lake clarity was the most important factor affecting end-of-season mixing depth in small Shield lakes.

Résumé : Dans une étude de 3 ans de 21 lacs du nord de l'Ontario, nous avons mis au point des modèles qui déterminent la durée de la couverture de glace et qui prédisent le calendrier de la stratification du printemps et la profondeur de la couche de brassage en fin de saison. Le modèle qui détermine les dates de gel et de dégel se base sur la variabilité journalière de la température de l'eau mesurée sur des enregistreurs de données suspendus à 1 et 2 m sous la surface de l'eau. Le modèle qui prédit la durée de l'intervalle de temps (jours) entre le dégel du lac et sa stratification est basé sur la température moyenne de l'air en mai (degrés Celsius), le carbone organique dissous et la surface du lac (r^2 = 0,79). La profondeur de la couche de brassage en fin de saison peut être mieux prédite à partir de l'intervalle entre le dégel et la stratification et de la concentration de carbone organique dissous (r^2 = 0,72). En utilisant un modèle simple de scénario de changement climatique, nous avons réussi à montrer que la température de l'air accrue, plus que la clarté plus grande de l'eau, est le facteur le plus important à affecter le calendrier de la stratification. En revanche, la clarté du lac est le facteur le plus important qui affecte la profondeur de la couche de brassage en fin de saison dans les petits lacs du Bouclier canadien.

[Traduit par la Rédaction]

Introduction

Climate change is expected to have major effects on the timing and duration of ice cover in north-temperate lakes (Magnuson et al. 2000; Gitay et al. 2001; Lofgren et al. 2002), with important implications for the biology of these systems (De Stasio et al. 1996; Magnuson et al. 1997; King et al. 1999). The four most widely used climate models predict that the most significant air temperature increase in the Great Lakes region of North America will occur in the winter (increase of 4–9 °C) and spring (3–8 °C), bringing about much earlier loss of ice cover (Mortsch and Quinn 1996). However, to date, most of the climate change studies dealing with lakes have focused on late-summer conditions, particularly the effects on hypolimnetic waters where important cold-water species such as lake trout (*Salvelinus namaycush*) exist.

The biological importance of the end-of-season thermocline structure or mixing depth is significant because many sport fish, such as lake trout, are highly dependent on thermally defined niches (Schindler 2001). Lake trout optimal temperature range is from 10 to 12 °C (Ryan and Marshall 1994). Increased lake clarity has been shown to increase meta- and hypo-limnetic water temperatures, which may

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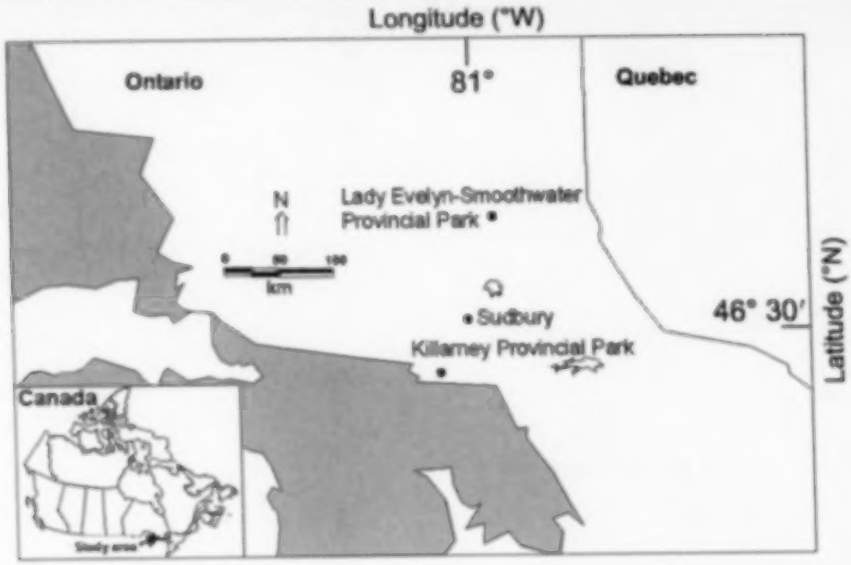
Fig. 1. The three areas in northeastern Ontario where the study lakes are located.



have significant ramifications for cold-water species such as opossum shrimp (*Mysis relicta*), cisco (*Coregonus artedi*), and lake whitefish (*Coregonus clupeaformis*) (Schindler et al. 1996; Yu and Culver 2000). Dillon et al. (2003) predicted a decrease of 8%–100% in optimal habitat for lake trout following a 2-m increase in transparency owing to decreased dissolved organic carbon (DOC) concentrations, while Shuter and Lester (2004) predicted a decrease in the diversity of prey available to adult and young lake trout.

In contrast, there has been relatively little attention paid to the effects that climate warming will have on lakes during the period from ice-out to the formation of the thermocline. Declines in primary productivity in the Laurentian Great Lakes have been predicted as a result of a warmer climate and longer period of stratification (Brooks and Zastrow 2002). However, Petterson et al. (2003) have shown that the warmer climatic conditions result in higher primary productivity. Given the discrepancy between these studies, models that predict the timing of lake stratification at a smaller scale will help expand our current understanding of potential climate change effects. Lastly, thermal inputs during this period are thought to be critical to the timing of biological cycles and the end-of-year thermal structure (De Stasio et al. 1996; King et al. 1997; Snucins and Gunn 2000). Models that predict the timing of lake stratification using parameters related to climate change such as DOC or water clarity would therefore be useful (Fee et al. 1996; Magnuson et al. 1997).

Accurately determining lake thaw for remote lakes is a necessary step in building better models of climate change effects for application across lake regions. Ice phenology can be predicted using air temperature records at local meteorological stations (Walsh et al. 1998; Fang and Stefan 1999). Weather stations, however, are often sparsely distributed in northern areas, making it difficult to predict ice phenology. Models based on air temperature records used to predict lake thaw over such wide areas may not be as accurate because ice phenology is greatly affected by variations in lake ice condition (Heron and Woo 1994) and local lake characteristics such as elevation, surface area, depth, and wind exposure (Stewart and Haugen 1990; Gao and Stefan 1999). Eyewitness accounts (Stewart and Haugen 1990; Futter 2003) and satellite photographs (Wynne et al. 1996) can also be used to determine ice cover, but observational data are usually limited to road-accessible lakes, while satellite images can be severely affected by cloud cover. The resolution of satellite images may also be limited by the frequency of the flight paths over an area.

Lake clarity is predicted to increase in many lakes with climate change because of reduced inputs of coloured DOC from drier watersheds (Fee et al. 1996; Schindler et al. 1996; Magnuson et al. 1997). Ultraclear lakes in the Killarney area have already shown increased clarity in recent decades (Gunn et al. 2001).

In small Precambrian Shield lakes, DOC is one of the primary controllers of UV light and visible light attenuation (Scully and Lean 1994; Fee et al. 1996) as well as the end-of-season thermal structure in lakes (Pérez-Fuentetaja et al. 1999; Snucins and Gunn 2000). For example, Snucins and Gunn (2000) found that during warm years, clear lakes (DOC < 2 mg·L^{-1}) had marked warming of the surface water and a deepening of the thermocline, resulting in a decrease in the amount of cold-water habitat for certain fish species. More surprising was the observation that coloured lakes (DOC > 4 mg·L^{-1}) exhibited an increase in cold-water habitat in warm years. One hypothesis for this unexpected finding was that rapid warming of coloured lakes (i.e., with higher DOC) resulted in earlier stratification that captured more cold water below the thermocline.

This study was designed to determine factors influencing (*i*) ice cover duration on remote study lakes, (*ii*) the timing of the spring stratification, and (*iii*) the end-of-summer mixing depth. Regression models were developed from data collected from 21 lakes during the spring and summer of 2002

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Table 1. Physical and chemical characteristics of the study lakes.

	Minimum	Median	Maximum
Surface area (ha)	3.4	131.5	795.2
DOC (mg·L^{-1})	0.1	2.4	6.5
Mean depth (m)	2.7	9.0	20.5
Maximum depth (m)	5.0	23.8	61.0
Fetch (km)	0.2	1.0	2.8
Elevation (m)	182	258	457
Secchi depth (m)	2.2	9.5	31

Note: DOC, dissolved organic carbon.

and 2003 and then tested using data collected from 53 lakes in 1997 and from 46 lakes in 2003.

Methods

Study area

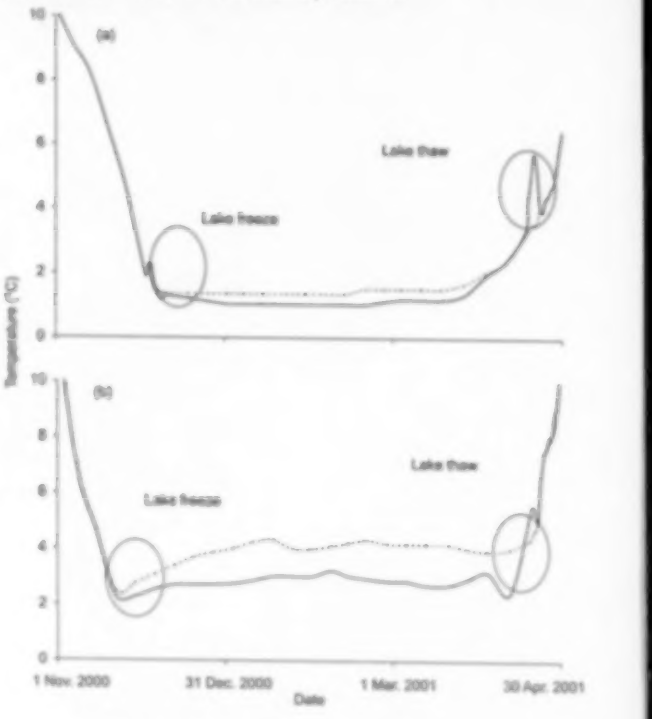
All study lakes were clustered at three locations in northeastern Ontario: (*i*) within Killarney Provincial Park, (*ii*) within the City of Greater Sudbury, and (*iii*) in Lady Evelyn Smoothwater Provincial Park (Fig. 1). These three areas provided an elevation range of 182–457 m above sea level and a latitude range of 46°01′00″N to 47°23′00″N (precise to the nearest 5″). Latitude, longitude, and elevation of the three clusters were correlated. Killarney contained the most southerly and westerly lakes that were also at the lowest elevation. The Lady Evelyn Smoothwater lakes were the most northerly, easterly lakes and had the highest elevations, whereas the Sudbury lakes were intermediate in geographic location and elevation. Study lakes varied widely in DOC concentration (0.1–6.5 mg·L^{-1}), Secchi depth (2.2–31.0 m), surface area (3.4–795.2 ha), maximum depth (5.0–61.0 m), and fetch (0.2–2.8 km) covering a broad range of the lake characteristics for the Boreal Shield region of Canada (Table 1). In 2002, meteorological stations were established at each of the three regions to record air temperature using a StowAway® TidbiT® model TBI32-05+37 data logger (Onset Computer, Bourne, Massachusetts).

Determining ice cover duration

Continuous-recording data loggers were also used to measure ice-on and ice-off dates based on the assumption that the daily variability in surface temperatures would reflect the occurrence of wind-driven surface mixing and thus the presence–absence of an ice cover. During the fall of 2000, temperature loggers were suspended 1 and 2 m below the water surface from float lines in 21 lakes. The float line was placed approximately in the middle of the main basin of each lake near the site of maximum depth. Data loggers were programmed to record water temperature on an hourly basis from mid-October to mid-May. Prior to deployment, each temperature logger was calibrated with a National Bureau of Standards thermometer over a temperature range of 0.5–23.4 °C and found to be accurate within ±0.2 °C. After removal from the field in spring 2001 and 2002, the loggers were again checked against a National Bureau of Standards thermometer to ensure that no significant drift had occurred over the field season.

The measured changes in daily variability of water temperatures at 1 and 2 m deep (Fig. 2) were used to develop a model to predict freeze and thaw dates. Freeze date was defined as the first day that the lake had a covering of ice on the main basin that did not thaw for at least 3 days. Thaw date was defined as the first day on which the main basin of the lake was ice free. An initial version of the model was developed using water temperature records from 16 lakes in the winter of 2001. Preliminary validation of the model was provided by visual observations at two lakes, Ramsey and Bethel Lakes (Fig. 2). In the winter of 2002, visual observations of freeze and thaw dates were available for an additional seven lakes, bringing the total to nine (Fig. 3).

Fig. 2. Daily average water temperature at 1 m (solid line) and 2 m (broken line) below the surface for (*a*) Ramsey and (*b*) Bethel Lakes located in Sudbury, Ontario.



A preliminary examination of temperature plots for Ramsey Lake in Sudbury (Fig. 2) showed that variability of water temperature at 1 m decreased after observed lake freeze and then increased substantially after the observed date of lake thaw. In the following winter (2002), this preliminary model was tested with water temperature data for 24 lakes and visual records maintained at nine accessible Sudbury lakes. In the final year (2003), the ice-on/off model was used to determine ice cover in 11 remote Killarney lakes and visual records were maintained at 10 accessible Sudbury lakes.

Statistical analysis

We predicted freeze date by estimating the day on which changes in water temperature stabilized after ice formation. This reflected a lack of wind-induced mixing. For freeze dates, we defined stability in water temperature as the 5-day variance in average daily water temperatures. Freezing was predicted to have occurred when the 5-day variance dropped

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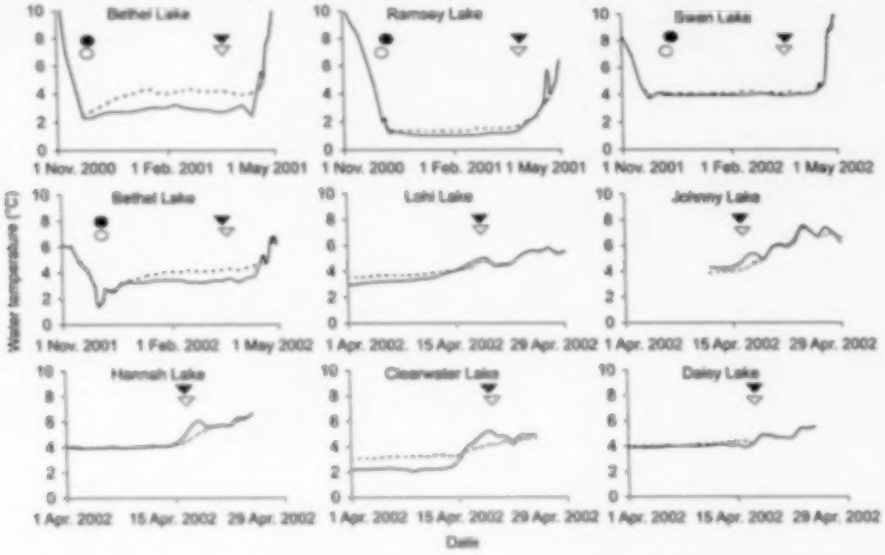
Fig. 3. Observed and predicted freeze and thaw dates for the nine study lakes that were accessible for visual confirmation of ice events. The solid line represents water temperature at 1 m below the surface, the broken line represents water temperature at 2 m below the surface, the closed circle is the observed freeze date, the open circle is the predicted freeze date, the closed triangle is the observed thaw date, and the open triangle is the predicted thaw date.



to a stable minimum. The target stable minimum variance was determined by estimating the 5-day variance that minimized the sum of squared differences between observed and modelled freeze dates.

Water temperature variability was greater during the period of spring thaw. For this reason, we predicted lake thaw date as the date on which the variance in hourly water temperature measurements over a 24-h period exceeded a critical variance threshold. The critical variance threshold was estimated by performing an iterative nonlinear least squares regression. The critical variance threshold is the target 24-h variance in water temperature that minimized the sum of squared differences between observed and predicted lake thaw dates.

Determining the date of lake stratification and end-of-season mixing depth

Twenty-one study lakes were used for this portion of the study. Ten lakes were located in Sudbury and 11 were more remotely located in Killarney Provincial Park. The lakes were selected based on accessibility, range in clarity (DOC = 0.9–5.9 mg·L^{-1}), and morphological features (elevation 189–280 m above sea level, surface area 6.1–795.2 ha, maximum depth 7.5–61.0 m). Visual observations for the 10 locally accessible Sudbury lakes were used to establish thaw dates in 2002 and 2003. For the 11 remote lakes located within Killarney Provincial Park, lake thaw dates were estimated using the water temperature data.

Temperature profiles were measured biweekly at 0.25-m intervals using a YSI meter (model 52) (Fisher Scientific, Unionville, Ontario) in the 21 lakes from 29 April to 7 June 2002 and from 28 April to 27 May 2003 to determine stratification date. End-of-season temperature profiles were measured in the final week of August in 2002 and 2003 following Snucins and Gunn (2000) (near maximum depth, 1-m interval). Each YSI meter was calibrated against a National Bureau of Standards thermometer over a temperature range of 0.1–25 °C.

Secchi disc depth transparencies for each lake were measured twice weekly. DOC samples were collected weekly as composite samples of the water column to a depth of two times Secchi disc depth + 1 m. A Cary 100 Bio UV-Visible spectrophotometer (Varian Canada Inc., Mississauga, Ontario) was used to estimate the DOC concentration (milligrams per litre) from a regression relationship (r^2 = 0.94) developed by Beaudclerc and Gunn (2001) between DOC and the absorbance of the water sample at 320 nm. DOC was the primary factor controlling Secchi disc depth (r^2 = 0.62), and DOC remained constant for each lake throughout the spring sampling period.

To deal with the problem of multiple unstable stratification events, we defined stratification to have occurred when temperature changed ≥1 °C and density changed ≥0.05 kg·m^{-3} over 1-m depth intervals (Vallentyne 1957; Pérez-Fuentetaja et al. 1999). The number of days to stratification (DTS) was then calculated as the difference between the Julian date of lake thaw and the Julian date of lake stratification.

Statistical analysis

Simple and multiple regressions were employed to determine which parameters were significantly correlated with DTS. The parameters used for modeling DTS included May mean air temperature, elevation, latitude, mean depth, maximum depth, and surface area. All variables were tested and were found to be normally distributed, with the exception of latitude and lake area; these two parameters were log-transformed prior to analysis.

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Spearman's rank correlation analysis was used to select the best combination of independent variables for use in a standard stepwise multiple linear regression. Included in the Spearman's correlation analysis was DTS, mean May air temperature (MAY T), lake area (AREA), the log of lake area (LOG AREA), maximum depth (MAX D), mean depth (MEAN D), spring Secchi depth (SECCHI), spring total chlorophyll concentration (CHLORO), spring DOC, mean June, July, and August air temperature (JUNE T, JUL T, and AUG T), and mean air temperature for June, July, and August combined (AVG T). The same method was used to assess the factors that affect the end-of-season thermal structure, specifically the mixing depth, using the predicted stratification date as one of the potential variables. The models were tested on the end-of-season mixing depths of the 53 lakes surveyed in 1997 by Snucins and Gunn (2000) and 46 of the same lakes resurveyed in 2003 (J. Gunn, unpublished data). We compared our best model and Snucins and Gunn's (2000) model based on DOC alone to determine which model better predicted end-of-season mixing depth. To do this, we used the data from the 46 lakes surveyed in 2003 and developed a correlation between end-of-season mixing depth and DOC. We then compared this relationship and our best model with the 1:1 line of predicted versus observed end-of-season mixing depth from the 1997 lake data set. Finally, our strongest model for predicting DTS was then used to examine the potential effects of climate change such as increased air temperature or declines in lake clarity because of drought effects on DOC export from catchment areas.

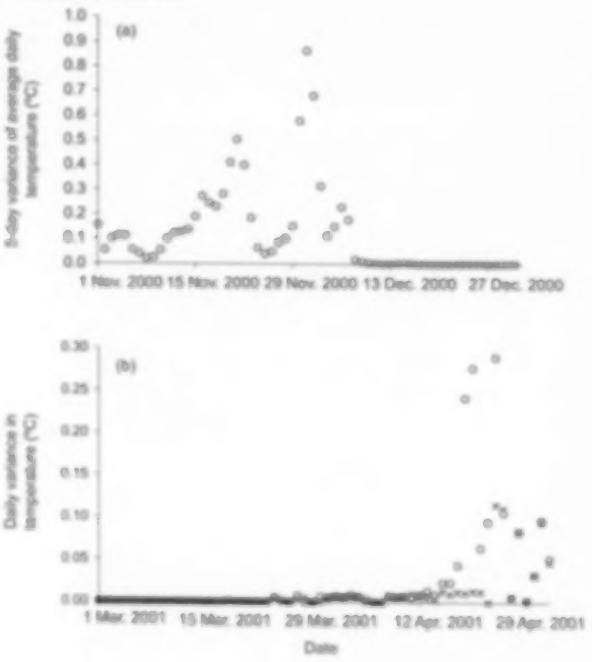
Results

Determining ice cover

The dates of lake freeze and lake thaw can be predicted within ±2 days using continuous water temperature data at 1 and 2 m below the water surface (Fig. 3). Lakes are predicted to have frozen for the winter season when the variance of the 5-day average of the daily average water temperature at 1 m is less than 0.026 °C (Fig. 4*a*). Lake thaw is predicted to have occurred when average daily water temperature at both 1 and 2 m depths is greater than 4 °C and the variance of the average daily water temperature at both 1 and 2 m is greater than 0.065 °C (Fig. 4*b*). The "greater than 4 °C" criterion prevents the model from predicting thaw during the periods of heating through spring-time black ice. In addition, because there can be heating through black ice, it was necessary to use the water temperature at the 2 m depth to moderate the effect of prethaw heating.

In the winter of 2000–2001, the dates over which the 21 lakes were predicted to have frozen spanned approximately 1 month from 18 November 2000 to 13 December 2000. The thaw period for the same winter was much shorter, with predicted dates ranging from 17 April to 30 April 2001. For the winter of 2001–2002, the predicted freeze dates (12 November 2001 to 16 December 2001) and thaw dates (16 April to 28 April 2002) were similar to those for 2000–2001. Predicted and observed thaw dates for the winter of 2002–2003 were later than for the previous years and extended from 23 April to 3 May 2003. Predicted thaw dates from winter 2002, the only year that air temperature was recorded at each location, were significantly correlated ($r = 0.92$, df = 20, $p < 0.0001$) with cumulative thaw degree-days.

Fig. 4. (*a*) Five-day variance of the average daily temperature at 1 m below the water surface for Ramsey Lake (November–December 2000). (*b*) Daily variance in water temperature at 1 m (○) and 2 m (×) below the water surface for Ramsey Lake (March–April 2001).



Date of lake stratification

Three variables, May mean air temperature (degrees Celsius), DOC (milligrams per litre), and lake area (hectares), emerged as significant in the following multiple regression analysis ($p < 0.01$, df = 3, 38):

$$(1) \quad \text{DTS} = -5.87(\text{MAY T}) - 3.35(\text{DOC}) + 0.01(\text{AREA}) + 91.24 \qquad (r^2 = 0.79)$$

The Pearson product-moment correlation coefficient (r) between May air temperature and DTS was –0.84, for DOC, it was 0.19, and for area, the correlation was –0.34. This phenomenon is best explained by Legendre and Legendre (1998) where a negative fraction simply indicates that the two variables together explain the effect better than taking the sum of the two individual effects of these variables. The absolute value of explained variability is greater than 100% because two of the variables (DOC and temperature) have opposing effects on DTS but are also interacting with each other (warmer temperatures and lower DOC were observed in 2003 and cooler temperatures and higher DOC were observed in 2002). For these reasons, the ability to explain the variability in the response variable (DTS) is lowered because the explanatory variables (DOC and air temperature) have strong opposing effects on the response variable (DTS) and are strongly correlated with each other (Legendre and Legendre 1998).

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Table 2. Spearman's rank correlations for dependent and independent variables for all lakes (n = 42) from 2002 and 2003.

	MIX D	DTS	AREA	LOG AREA	MAX D	MEAN D
DTS	0.39*					
AREA	0.28	0.28				
LOG AREA	0.28	0.28	1.00**			
MAX D	0.47**	0.32*	0.71**	0.71**		
MEAN D	0.46**	0.27	0.54**	0.54**	0.91**	
SECCHI	0.59**	0.45**	0.25	0.25	0.66**	0.67**
CHLORO	–0.56**	–0.14	–0.12	–0.12	–0.45**	–0.53**
DOC	–0.72**	–0.16	–0.09	–0.09	–0.48**	–0.52**
MAY T	0.06	–0.76**				
JUNE T	0.04	0.64**				
JUL T	0.05	0.64**				
AUG T	0.04	0.71**				
AVG T	0.05	0.64**				

Note: The mean air temperatures for the month of May (MAY T), June (JUNE T), July (JUL T), and August (AUG T) were analysed in addition to the combined mean air temperature for June, July, and August as one parameter (AVG T). Significant correlations are indicated by * ($p < 0.05$) and ** ($p < 0.01$). MIX D, mixing depth; DTS, days to stratification; AREA, lake area; LOG AREA, the log of lake area; MAX D, maximum depth; MEAN D, mean depth; SECCHI, spring Secchi depth; CHLORO, spring total chlorophyll concentration; DOC, dissolved organic carbon.

End-of-season mixing depth

The end-of-season mixing depth (MIX D) is often used to define thermal habitat of many species. Habitat volumes for cold-water species are at their minimum at the end of the summer (Clark et al. 2004). The end-of-season mixing depth was significantly correlated with many physical and optical lake characteristics; however, it was not correlated with air temperature (Table 2). From these parameters, one multiple regression model was developed ($p < 0.01$, df = 2, 39) to predict the end-of-season mixing depth using DOC and DTS (eq. 2). All of the physical characteristics were nonsignificant whenever incorporated into a regression model with DOC:

$$\text{MIX D} = 0.04(\text{DTS}) - 1.25(\text{DOC}) + 9.65 \quad (r^2 = 0.72) \tag{2}$$

Model testing

In the winter of 1996, DOC measurements (0.1–6.5 mg·L^{-1}) were taken on 53 lakes in Killarney Provincial Park. In 1997, the mean May air temperature was 7.4 °C and end-of-season temperature profiles were measured for each lake. Using this information, we calculated DTS from eq. 1 for each of these lakes and then used eq. 2 to predict end-of-season mixing depth. Our model was able to explain 73% of the variability in end-of-season mixing depth (Fig. 5*a*), the same procedure was used with the 2003 data set (46 lakes) with similar results ($r^2 = 0.66$) (Fig. 5*b*).

We could explain 73% of the end-of-season mixing depth using DOC as the sole predictor; however, when tested against 53 lakes surveyed in 1997 using eq. 2 with both DTS and DOC as variables, a better 1:1 relationship between predicted and observed mixing depth was observed (Fig. 6).

Model application

The second-generation atmospheric general circulation model from the Canadian Climate Centre of Environment Canada (CCC GCMII) simulated an increase in northern Ontario summer air temperatures of 4–5 °C and an increase in winter air temperatures of 6–7 °C with a change from present CO_2 levels to doubled CO_2 concentrations in the atmosphere (Magnuson et al. 1997). We used a simple climate change scenario with the developed models to determine the effect of temperature and DOC on DTS, knowing that mean May air temperature was 7.9 and 8.8 °C in 2002 and 11.3 and 11.4 °C in 2003 in Killarney and Sudbury, respectively. In these scenarios, selected mean May air temperatures of 8, 10, and to 12 °C and DOC concentrations of 1, 3, and 5 mg·L^{-1} were used to predict DTS for a model lake with a surface area of 130 ha (the average area for the 21 study lakes). The scenario for clear lakes with a DOC of 1 mg·L^{-1} suggests that an increase from the current 10 °C to 12 °C would result in a decrease in DTS from 30 to 19 days (Table 3). The climate change scenario suggests a similar influence on the higher DOC lakes, decreasing DTS from 17 to 5 days (Table 3). A 2 °C increase, from 10 to 12 °C, advances stratification by approximately 12 days in all lakes with DOC less than 5 mg·L^{-1}.

The model developed to predict the end-of-season thermocline depth (eq. 2) was used to also predict potential changes under different climatic conditions. In this case, DTS was varied (5, 10, 20, and 30 days) and the DOC was also varied (1, 3, and 5 mg·L^{-1}). A change in DTS from 5 to 30 days resulted in a difference in end-of-season thermocline depth of less than 1 m, whereas a decrease in DOC levels from 5 to 3 mg·L^{-1} or from 3 to 1 mg·L^{-1} resulted in a deeper mixing depth by 2.5 m (Table 4).

Discussion

Using our approach and model, the timing of ice cover formation and loss can be easily monitored through an annual visit during the ice-free season to install and retrieve data from temperature data loggers. These findings will therefore have important practical applications for numerous monitoring programs and should lead to an improved understanding of global climate change effects on Precambrian Shield lakes.

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Fig. 5. (*a*) Predicted versus observed end-of-season mixing depth (MIX D) (r^2 = 0.73) for 53 lakes from 1997 using the model generated from spring 2002–2003 data from 21 lakes (95% confidence limits are shown by broken lines), where MIX D = 0.04(DTS) – 1.25(DOC) + 9.65 and where days to stratification (DTS) = –5.87(MAY T) – 3.35(DOC) + 0.01(AREA) + 91.24. (*b*) Predicted versus observed end-of-season mixing depth (r^2 = 0.66) for lakes from 2003 using the model generated from spring 2002–2003 data from 21 lakes (95% confidence limits are shown by broken lines), where MIX D = 0.04(DTS) – 1.25(DOC) + 9.65 and where DTS = –5.87(MAY T) – 3.35(DOC) + 0.01(AREA) +91.24. DOC, dissolved organic carbon.

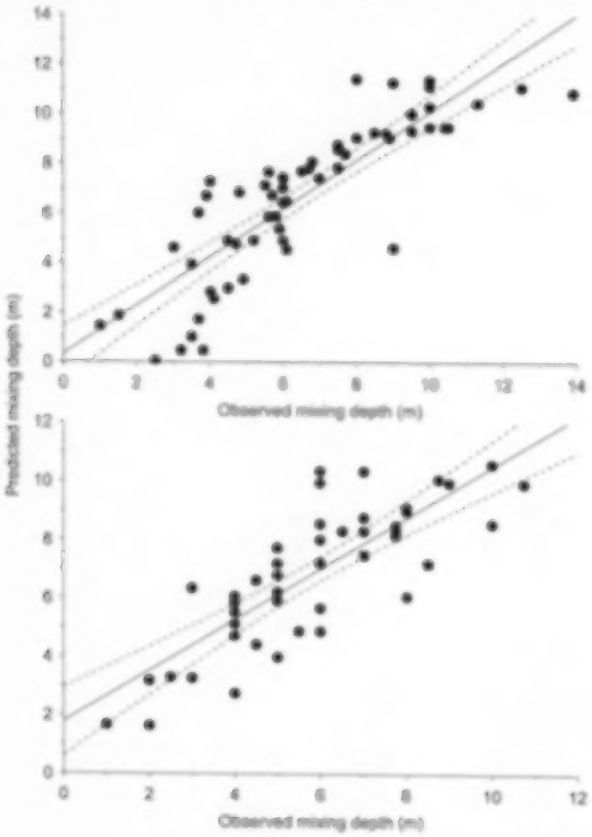
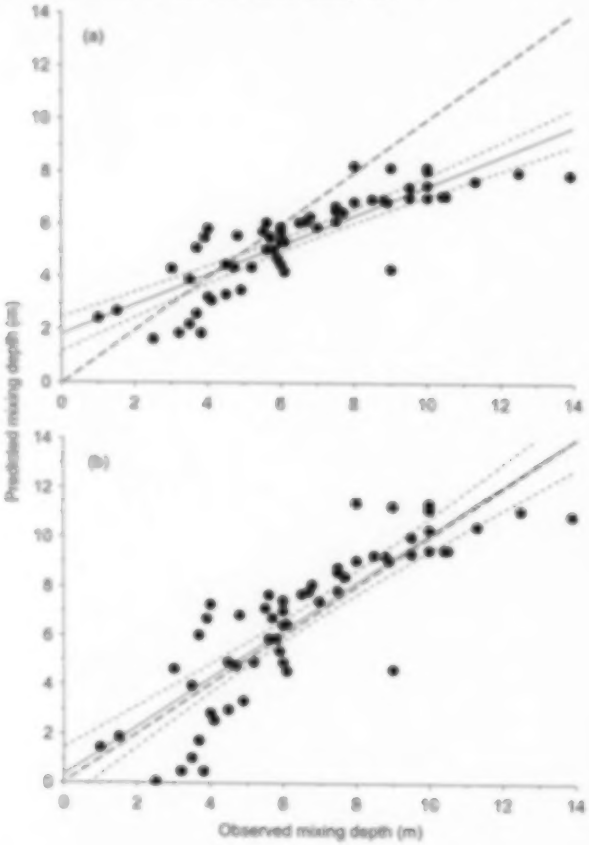


Fig. 6. (*a*) Predicted versus observed end-of-season mixing depth (r^2 = 0.73) for lakes surveyed in 1997 using the dissolved organic carbon (DOC) and end-of-season mixing depth (MIX D) relationship developed from the 2003 lake survey of 46 lakes. The trend line (solid line) and the 95% confidence limits (broken lines) are not parallel to the 1:1 line (long-dashed line). (*b*) Predicted versus observed end-of-season mixing depth (r^2 = 0.73) for lakes surveyed in 1997 using the model generated from spring 2002–2003 data from 21 lakes, where MIX D = 0.04(DTS) – 1.25(DOC) + 9.65 and where days to stratification (DTS) = –5.87(MAY T) – 3.35(DOC) + 0.01(AREA) + 91.24. The trend line (solid line) and the 95% confidence limits (broken lines) follow the 1:1 line (long-dashed line). This model is a better predictor of end-of-season mixing depth.



Although cumulative thaw degree-days from air temperature records were highly correlated with our predicted thaw days using under ice data loggers, our model based on water temperature records is a more accurate predictor of freeze–thaw events. Lakes in the same area, with the same cumulative thaw degree-days, had very different thaw dates because of differences in factors such as lake area and depth that affect heat content and wind exposure (Vavrus et al. 1996; Wynne et al. 1996). These factors would not be accounted for using only air temperature measurements.

Warmer air temperature, higher DOC concentration, and smaller lake area all result in a shorter time to the onset of lake stratification, supporting the theories presented by Schindler (1971), France (1997), Fee et al. (1996), and Snucins and Gunn (2000). Warmer air temperatures led to more rapid heating of lake surface water causing a change in density sooner, while a smaller lake would be exposed to less wind and therefore less mixing of the surface waters and bottom water, allowing stratification to proceed more rapidly. A coloured lake stratifies quicker than a clear lake because solar radiation is absorbed more readily in the surface waters of coloured lakes.

Although the timing of lake stratification (DTS) was significantly correlated with the end-of-season mixing depth, differences in DOC concentration among lakes were the far more important determinants of the end-of-season mixing depth. Clear lakes had much deeper mixing depths at the end

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Table 3. Predicted number of days for a lake to stratify under various climatic scenarios (i.e., change in May air temperature (MAY T) and (or) dissolved organic carbon (DOC) concentration) using the model developed where days to stratification (DTS) = –5.87(MAY T) – 3.35(DOC) + 0.01(AREA) + 91.24.

May mean air temperature (°C)	DOC ($mg \cdot L^{-1}$)	Lake area (ha)	Predicted DTS
8.0	1.0	130.0	42
10.0	1.0	130.0	30
12.0	1.0	130.0	19
8.0	3.0	130.0	36
10.0	3.0	130.0	24
12.0	3.0	130.0	12
8.0	5.0	130.0	29
10.0	5.0	130.0	17
12.0	5.0	130.0	5

of the season than coloured lakes. These findings are similar to the Mazumder and Taylor (1994) study, where in small lakes, lake clarity was found to be the primary predictor of end-of-season mixing depth followed by lake size (wind effect). These results are further supported by the works of Pérez-Fuentetaja et al. (1999) and Snucins and Gunn (2000). The explained variances from our end-of-season mixing depth model were similar to the results of Snucins and Gunn (2000) using DOC as the sole predictor of mixing depth; however, our end-of-season mixing depth model was a better predictor of the actual measured thermal conditions.

Earlier lake thaw dates and increased air temperatures would shorten the time to the onset of stratification, whereas a decline in DOC has been observed to prolong the onset of the spring stratification period. However, the simulated climate scenarios that we used demonstrate that small changes in temperature have a stronger influence than large changes in lake clarity on the amount of time for a lake to stratify. In our scenario, an increase in air temperature of 2 °C resulted in a shorter spring stratification period by 11–12 days, while a 50% reduction in DOC concentration from 4 to 2 $mg \cdot L^{-1}$ only increased the onset of spring stratification by 5–6 days.

Conversely, lake clarity, specifically DOC, not DTS (the length of the spring stratification period), emerged as the best explanatory variable for the end-of-season mixing depth. While DTS, which primarily reflects spring air temperature, was correlated with the end-of-season mixing depth, the climate change scenarios in this study revealed that if we shortened the time from ice-out to stratification by 25 days, the mixing depth would be reduced by only 1 m, while a decrease in DOC of 2 $mg \cdot L^{-1}$ would result in deepening of the mixing depth by 2 m. A difference of 1–2 m in mixing depth may be unimportant in large, deep lakes where the total hypolimnetic volume would remain relatively unchanged; however, clear, shallow lakes are most at risk, where a change of 2 m in mixing depth could dramatically affect the hypolimnetic volume of the lake.

In this study, thermal habitat boundaries were defined using climate-affected parameters. This study demonstrates the importance of lake clarity and air temperature in the development of the thermocline and the end-of-summer mixing depth in small Shield lakes. Although both air temperature and lake clarity influence thermocline formation and depth, air temperature plays a larger role in determining when a lake will stratify, while lake clarity is more dominant in determining the end-of-season mixing depth. Increased lake clarity results in longer spring stratification periods (DTS) and deeper end-of-season mixing depth, whereas increased air temperature results in shorter spring stratification periods (DTS) and shallower mixing depths.

Table 4. Predicted effects of changes in the rate at which a lake stratifies and (or) changes in dissolved organic carbon (DOC) concentrations occur on the end-of-season mixing depth (depth from the surface to the thermocline).

DTS[a]	DOC ($mg \cdot L^{-1}$)	Predicted end-of-season mixing depth (m)
5	1.0	8.6
10	1.0	8.8
20	1.0	9.2
30	1.0	9.6
5	3.0	6.1
10	3.0	6.3
20	3.0	6.7
30	3.0	7.1
5	5.0	3.6
10	5.0	3.8
20	5.0	4.2
30	5.0	4.6

Note: The climate change scenarios use the model MIX D = 0.04(DTS) – 1.25(DOC) + 9.65.

[a]Days to stratification.

With the changing climate, the predicted antagonistic effects of increasing lake clarity and air temperature on the length of the spring stratification period (DTS) and end-of-season mixing depth will need to be closely monitored. Both lake clarity and air temperature have changed and will likely continue to change dramatically. It seems certain that the influence of these two abiotic factors on the physical environment will have significant impacts on the biota that live within the Shield environment. The simple models developed in this paper should be applicable to those Precambrian Shield lakes that are less than 800 ha and can extend our current knowledge of the timing of limnological events that have many important implications for the biota.

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Classical individual assignments versus mixture modeling to estimate stock proportions in Atlantic salmon (*Salmo salar*) catches from DNA microsatellite data

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Abstract: Mixture modeling is shown to outperform classical individual assignments for both estimating stock composition and identifying individuals' sources in a case study of an eight-locus DNA microsatellite database from 26 Atlantic salmon (*Salmo salar*) stocks of the Baltic Sea. Performance of the estimation methods was compared using self-assignment tests applied to each of the baseline samples and using independent repeat samples from two of the baseline stocks. The different theoretical underpinnings, hypothesis testing versus decision theory, of the methods explain their estimation capacities. In addition, actual catch samples from three northern Baltic Sea sites in 2000 were analysed by mixture modeling, and estimated compositions were consistent with previous knowledge. Baltic main basin and Gulf of Finland stocks were each minor components (<1% at any site), and three groups of Gulf of Bothnia stocks, wild (36%–43% among sites), Finnish hatchery (15%–49%), and Swedish hatchery (11%–41%), were each important with the two hatchery contributions trending geographically.

Résumé : Dans une étude de cas comportant une banque de données sur des microsatellites ADN à huit locus provenant de 26 stocks de saumons atlantiques (*Salmo salar*) de la Baltique, nous montrons qu'un modèle de mélange fonctionne mieux que les assignations individuelles classiques, tant pour estimer la composition des stocks que pour identifier les origines des individus. Nous avons comparé la performance des méthodes d'estimation à celle des tests d'auto-assignation appliqués à chacun des échantillons de base et à des échantillons répétées indépendants des deux stocks de base. Les différents fondements théoriques, évaluation d'hypothèse ou théorie de décision, des méthodes expliquent leur capacité d'estimation. De plus, nous avons analysé des échantillons réels de récoltes en 2000 dans trois sites du nord de la Baltique à l'aide de modèles de mélange; les compositions estimées que nous avons obtenues concordent avec nos connaissances antérieures. Les stocks du bassin principal de la Baltique et du golfe de Finlande sont tous deux des composantes mineures (<1 % à tous les sites) et trois groupes de stocks du golfe de Bothnie, stocks sauvages (36–43 % parmi les sites), stocks finlandais de pisciculture (15–49 %) et stocks suédois de pisciculture (11–41 %), sont tous importants; les deux contributions des pisciculteurs suivent des gradients géographiques.

[Traduit par la Rédaction]

Introduction

Genetic information has been used effectively for assessing stock composition of mixed catches to aid in management of the comprising fish stocks (Begg et al. 1999; Cadrin et al. 2005 and references therein). Genetic mixed-stock analyses have been applied, for example, to Pacific salmon to determine fishery openings and closures with a view to providing harvest benefits or meeting conservation needs, to address catch allocation and equity issues among user groups and between countries, to provide data for in-season run size updates, and to investigate migration patterns and timing (Shaklee et al. 1999). The data required are the counts of multilocus genotypes in the mixture sample and their estimated relative frequencies from so-called baseline samples for each of the separate potentially contributing stocks. (Hereafter, the term relative frequency will be abbreviated to frequency, and the latter will be used exclusively to refer to a probability and not to a count. Also, baseline stock refers to any of the sampled stocks.) The genotype frequencies are typically estimated from allele frequencies assuming that the Hardy–Weinberg and linkage equilibrium (HWLE) conditions hold. The allele frequencies themselves traditionally have been estimated by their observed proportions at a locus in a baseline sample. However, concerns of baseline sampling zeroes for alleles and their critical effects on ap-

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parent sources of mixture genotypes — a sampling zero without adjustment implies that the stock cannot be the source of a mixture genotype with the allele — have led to alternative Bayesian estimators of allele frequencies including the Rannala–Mountain formula (Rannala and Mountain 1997) and the Pella–Masuda shrinkage method (Pella and Masuda 2001).

In earlier years, the genetic information was mainly from allozymes, and the limited numbers of loci and few alleles per locus resulted in much shared probability mass, or overlap, of the genotype distributions among baseline stocks. However, nowadays, more diverse information on DNA-level variation, commonly from microsatellites, is also available. Microsatellite variation has already been used successfully for mixed-stock analysis of sockeye salmon (*Oncorhynchus nerka*) (Beacham and Wood 1999) and steelhead (*Oncorhynchus mykiss*) (Beacham et al. 1999) and by King et al. (2001) to demonstrate differences between European and North American Atlantic salmon (*Salmo salar*). The large number of loci and increased numbers of alleles per locus for microsatellites decrease the shared probability mass among baseline stocks. Nonetheless, the genotype distributions generally continue to be superimposed, and when the alternative Bayesian estimators of allele frequencies are used, all multilocus genotypes are estimated as potentially present in every baseline stock.

Two approaches to stock composition estimation are compared here: classical individual assignments and mixture modeling. In both, baseline samples are assumed to be available with which to estimate the frequencies of the genotypes in the separate stocks. First, in the classical individual assignments method, each mixture individual is assigned to the baseline stock in which the frequency of its genotype is the greatest (Cornuet et al. 1999; Luikart and England 1999). This step in the method is usually called an individual assignment test, which is indicative of a conceptual grounding in hypothesis testing theory (e.g., Davies et al. 1999; Guinand et al. 2004). Proponents commonly frame the individual assignment test in evocative terms of the theory such as ratios of likelihoods, whereby the genotype frequencies in the possible source stocks are compared via their ratios (Banks and Eichert 2000; Guinand et al. 2004). Many studies have involved individual assignment tests, including species such as polar bears (*Ursus maritimus*) (Paetkau et al. 1995), domestic dogs (*Canis familiaris*) (Koskinen 2003), North Atlantic redfish (*Sebastes* sp.) (Roques et al. 1999), and Atlantic salmon (King et al. 2001). The overall effect of the individual assignment tests is to partition the possible genotypes into assignment classes corresponding one-to-one with the baseline stocks such that each class is composed exclusively of the genotypes whose frequencies are highest in its associated stock. Regardless of assignments made of other mixture individuals, each mixture individual is assigned to the stock of its genotype's assignment class. Because their genotype distributions overlap, stocks include genotypes of incorrect assignment classes, and such individuals are misclassified to another stock in which these genotypes are most frequent. After the individuals have been assigned to the stocks from their genotypes, their genotype frequencies among stocks are not used further and their uncertain stock sources are treated as if known and correct. The stock composition of the mixture is estimated by the proportions assigned to the possible sources. Note, however, that the apparent stock composition from the assignments is biased by misclassifications and that the precision of this estimate will be overstated if the uncertain stock sources of the individuals continue to be treated as known (e.g., Pella and Masuda 2005, pp. 532–536). The extension of individual assignment tests to include stock composition estimation is implicit whenever individual assignment tests are applied to individuals from population mixtures with unknown composition, and it is explicitly described in Hansen et al. (2001), Potvin and Bernatchez (2001), and Guinand et al. (2004). In contrast with hypothesis testing as a conceptual basis for the individual assignments approach, the assignment of individuals to their source stocks under mixture modeling is grounded on statistical decision theory (e.g., Hand 1981; Hastie et al. 2001).

In mixture modeling, the genotypes in the mixture sample are viewed as having been drawn from the genotype distributions of the baseline stocks with the stock proportions explicitly included as unknowns to be estimated (e.g., see eq. 2 in Pella and Milner 1987). Under statistical decision theory, the assignment of mixture individuals is accomplished through Bayes' theorem of mathematical statistics (Pella and Masuda 2005). Bayes' theorem is used to compute the posterior source probabilities of the individuals from prior (to seeing their genotype) probabilities for the source stocks and their genotype frequencies in each of the source stocks. The appropriate prior source probabilities are the unknown stock proportions of the mixture, which must be estimated. The posterior source probabilities are specific to each genotype and fundamental for validly classifying or assigning mixture individuals to their source stocks. The posterior source probabilities for a mixture individual having a particular genotype, say X, equal the proportions of mixture individuals with that genotype that are contributed by each of the baseline stocks, viz.

$$P(i|X) = \frac{p_i f(X|i)}{\sum_{s=1}^{c} p_s f(X|s)}$$

$$i = 1,\ldots,c, \quad 0 \le p_i \le 1, \quad \text{and} \sum_{i=1}^{c} p_i = 1$$

Here, a total of c stocks compose the mixture with stock proportions $\boldsymbol{p} = (p_1,\ldots, p_c)$, and the frequency of genotype X in the ith stock is denoted by $f(X|i)$, $i = 1,\ldots, c$. The assignment rule with the lowest misclassification rate assigns the individual to the source stock i^* for which the posterior source probability is maximal (Pella and Masuda 2005), i.e., $P(i^*|X) = \max_i\{P(i|X),\ i = 1,\ldots, c\}$. This rule will be termed the maximum a posteriori (MAP) rule. Notice that if the mixture is composed of equal proportions from the source stocks, the stock for which the genotype frequency is greatest is the same as that for which the posterior source probability is maximal. In this special case, the classical individual assignments rule is optimal.

Two estimation methods for the stock proportions of the mixture model are compared: the standard conditional maximum likelihood (CML) method (Fournier et al. 1984; Millar

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1987; Pella and Milner 1987) and a more recent Bayesian method (Pella and Masuda 2001). Under the standard CML method, the estimates of the stock proportions maximize the mixture likelihood function from the counts of mixture genotypes given their frequencies in the baseline stocks. The genotype frequencies in the separate baseline stocks are estimated from the baseline samples, assuming HWLE conditions, and using the observed allele frequencies. Any sampling zeroes in baseline samples are treated as real zeroes. Nonlinear algorithms are required to iteratively search for the CML estimate of stock proportions, and one of these recipes, called the expectation maximization (EM) algorithm (see eqs. 4 and 5 in Pella and Milner 1987), can be seen to fractionally allocate each individual to the baseline stocks in proportion to the estimates of its posterior source probabilities. The estimated posterior source probabilities at convergence of the EM algorithm, which are computed from estimates of genotype frequencies in the baseline stocks and the CML estimates for the stock proportions, could be used with the MAP rule to assign individuals to the sources as entities instead of fractions. However, this possibility is not considered further here. Lastly, the uncertainty in the CML estimate of stock composition is evaluated by the bootstrap method, namely by generating empirical distributions of the CML stock composition estimates for many resampled baseline and mixture samples. The CML estimate and its bootstrap evaluation account for multinomial sampling variation in stock composition of the mixture sample, uncertainty in the genotype frequencies because of sampling error in estimates of allele frequencies, and uncertainty in sources of individuals because of overlap of genotype distributions among baseline stocks.

The Bayesian method uses Gibbs sampling to generate draws from the so-called posterior probability distribution for the unknown stock proportions and allele frequencies in the separate stocks at the loci defining the mixture genotypes (Pella and Masuda 2001). The posterior probability distribution for the unknowns should not be confused with the previously mentioned posterior source probabilities of the mixture individuals, which are functions of these unknowns. However, the posterior probability distribution of the posterior source probabilities can be and is generated during the Gibbs sampling, and its average values among stocks will later be used to advantage for assigning individuals to their sources. The Gibbs sampling requires running the estimation algorithm past a burn-in period affected by initial conditions, and subsequent samples are used to describe the desired probability distribution for the unknowns. The posterior probability distribution of the unknowns is determined with negligible error from a large number of draws after burn-in. During repeated sampling from the posterior probability distribution for the unknowns, the mixture individuals are stochastically assigned as entities to the baseline stocks with probabilities equal to the posterior source probabilities computed from current draws of the unknowns. The long-run average proportion of an individual's assignments to a particular baseline stock equals its average posterior source probability for that stock. Later, each mixture individual is assigned to the source stock whose average posterior source probability is a maximum. This assignment rule is another form of the MAP rule.

Recently, information on DNA microsatellite variation has become available for Baltic Sea stocks of Atlantic salmon (Säisä et al. 2005). Earlier, allozyme variation among the Baltic Sea stocks was surveyed (Koljonen et al. 1999) and used to estimate stock proportions in Baltic Sea catches with mixture modeling and the CML estimation method (Koljonen and McKinnell 1996; Koljonen and Pella 1997). The average mean heterozygosity is more than 10 times greater at microsatellite (0.704) than at allozyme loci (0.058) (Koljonen et al. 2002). The number of different alleles was generally only two or three at allozyme loci, whereas the number of alleles ranges from 8 to 32, with a mean of 17.8 alleles per microsatellite locus within Baltic salmon populations. Microsatellite loci with up to 50 different alleles have been reported in Atlantic salmon from the east coast of Canada (McConnell et al. 1997) and from 2 to 29 different alleles per locus in European Atlantic salmon populations (Nielsen et al. 1999; Norris et al. 1999). Such high allelic diversity at a sufficient number of loci can easily result in a greater number of possible multilocus genotypes than the actual numbers of individual fish composing the populations. Also, the high number of alleles can result in sampling zeroes, as the larger the number of alleles at a single locus, the more likely that not all of the alleles will be observed in the baseline sample even if they occur in the populations studied. Nondetection of rare alleles in the baseline sampling can occur so frequently that substantive estimation bias results unless adjustments are made.

The aims of this study are to compare the accuracy and precision of stock composition estimation by classical individual assignments, mixture modeling using the standard CML method, and mixture modeling using the Bayesian method. In addition, success in the more challenging task of assigning individuals to their sources is compared for two of the three approaches: classical individual assignments and mixture modeling using the Bayesian method. These comparisons will be used to choose the most efficient method for future Atlantic salmon catch analysis in the Baltic Sea area. However, the level of genetic differentiation among Baltic Sea Atlantic salmon stocks represents quite well that of the other salmonids, and so the study has wide relevance and responds to the plea for comparative analyses by Manel et al. (2005). Lastly, the utility and resolving power are described for the data set of eight microsatellite loci from the 26 Baltic salmon stocks, and the Bayesian method is used to estimate the stock composition of actual mixed catches of the year 2000 from Finnish fisheries in the northern Baltic Sea.

Material and methods

Sampling of fish

For the baseline data, tissue samples of 1708 fish were sampled from Atlantic salmon stocks of 25 rivers draining into the Baltic Sea (Table 1; Fig. 1). Because both the wild Tornionjoki River salmon and their hatchery derivative were included, the total number of baseline stocks was 26. The baseline data represent well all stocks with meaningful contributions to Baltic Sea fisheries. The baseline data set was the same as used for the Finnish Atlantic salmon catches in 2002 (Anonymous 2003) and also formed part of the data in Säisä et al. (2005). Test samples of two baseline stocks,

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Table 1. Attributes of the microsatellite data set for eight loci from Atlantic salmon (*Salmo salar*) of the Baltic Sea region: the baseline stocks, sampling year, country of origin, sample sizes of individual fish (N), average sample sizes assayed per locus ($\bar{N}$), baseline proportion, $(N/\Sigma N) \times 100\%$, and the main mode of propagation (wild or hatchery).

Stock	Sampling year	Country	N	$\bar{N}$	Baseline proportion (%)	Propagation
Tornionjoki wild	2000	Finland, Sweden	117	114.0	6.9	Wild
Tornionjoki hatchery	1997	Finland	119	111.0	7.0	Hatchery
Simojoki	1997	Finland	59	56.6	3.5	Wild
Iijoki	1997	Finland	61	59.6	3.6	Hatchery
Oulujoki	1997	Finland	59	56.4	3.5	Hatchery
Kalixälven	2002	Sweden	169	168.5	9.9	Wild
Luleälven	1995, 1997	Sweden	60	59.8	3.5	Hatchery
Byskeälven	1994	Sweden	77	73.4	4.5	Wild
Skellefteälven	1995, 1996	Sweden	51	45.4	3.0	Hatchery
Vindelälven	1993, 1995	Sweden	50	50.0	2.9	Wild
Umeälven	1995	Sweden	50	48.4	2.9	Hatchery
Lögdeälven	1995	Sweden	49	45.3	2.9	Wild
Ångermanälven	1995	Sweden	60	57.9	3.5	Hatchery
Indalsälven	1995, 1997	Sweden	64	63.8	3.7	Hatchery
Ljungan	1998	Sweden	51	49.0	3.0	Wild
Ljusnan	1999	Sweden	53	51.5	3.1	Hatchery
Dalälven	1999	Sweden	60	59.3	3.5	Hatchery
Emån	1999	Sweden	54	50.3	3.2	Hatchery
Mörrumsån	1999	Sweden	42	40.8	2.5	Wild
Neva	1997	Russia	60	56.6	3.5	Hatchery
Kunda	1996	Estonia	60	55.1	3.5	Wild
Keila	1997	Estonia	53	47.6	3.1	Wild
Pärnu	1997	Estonia	26	22.0	1.5	Wild
Gauja	1998	Latvia	70	65.0	4.1	Hatchery
Daugava	1996	Latvia	68	65.5	4.0	Hatchery
Venta	1996	Latvia	66	52.8	3.9	Wild
Total			1708	1625.2	100	

Neva (n = 50) and wild Tornionjoki salmon (n = 65), were available from earlier studies and had been drawn in a different year than the current baseline samples. These additional samples will be called repeat baseline test samples. Neva test salmon were expected to provide a relatively easy identification task, as the stock is quite distinctive among the baseline stocks, although it is not the most unique. Identifying wild Tornionjoki test fish was expected to be a difficult task because of genetic similarity to their derivative hatchery stock.

Actual catch samples were available from the Finnish salmon fishery in 2000 (Fig. 1; Table 2), and their microsatellite genotypes were used to estimate stock composition using mixture modeling with the Bayesian estimation method. The fishery exploits returning adults that leave the Baltic Main Basin and migrate across the Åland Sea to spawn in northern rivers in Sweden and Finland. The samples were taken throughout the main fishing season at three important fishing areas off the Finnish coast that lie along the route of this northward migration. The lengths of the catch fish from each area were measured and daily scale samples were taken from a subsample stratified by size. Ages of the fish were determined by reading the scales and the DNA subsample was taken from the fish stratified by sea age (1–3 years) (Table 2).

Microsatellite DNA analysis

Total genomic DNA was extracted from the muscle tissue samples, adipose fins, or scales according to the method described in Taggart et al. (1992) or by using a QIAGEN DNeasy tissue sample kit (QIAGEN GmbH, Hilden, Germany). Variation was determined at eight microsatellite loci: *Ssa85* and *Ssa289* (McConnell et al. 1995); *Ssa171*, *Ssa197*, and *Ssa202* (O'Reilly et al. 1996); *SSOSL85* and *SSOSL417* (Slettan et al. 1995); and *SSOSL438* (Slettan et al. 1996).

Genotypes were assayed by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis, either with radioactive labeling and autoradiography as previously described by Koljonen et al. (2002) or with fluorescent labeling. In the fluorescent method, the forward PCR primer of each pair was labeled with the infrared dyes IRD-700 (*Ssa202*, *Ssa289*, *SSOSL85*, *SSOSL417*, and *SSOSL438*) and IRD-800 (*Ssa85*, *Ssa171*, and *Ssa197*). PCRs were performed in a 10-μL reaction volume with 15 ng of genomic DNA, 0.1 pmol of fluorescent-labeled primer, 5 pmol of each primer, 200 μmol dNTPs·L^{-1}, 10 mmol Tris–HCl·L^{-1} (pH 9.0), 1.5 mmol $MgCl_2$·L^{-1}, and 0.25 U (1 U ≈16.67 nkat) of *Taq* DNA polymerase (GE Healthcare, Helsinki, Finland). PCR products were separated on 25-cm 7% denaturing polyacrylamide gels and detected using a LI-COR automated DNA sequencer (LI-COR, Inc., Lincoln, Ne-

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Fig. 1. Map of the Baltic Sea with the locations of Atlantic salmon (*Salmo salar*) rivers and the three catch sampling sites indicated (asterisks).

Table 2. Site, latitude, calendar days of sampling, calendar week, International Council for the Exploration of the Sea (ICES) statistical area, sample sizes of DNA samples (DNA *N*), and catch scale samples (scales *N*) from the Finnish Atlantic salmon (*Salmo salar*) catches in the year 2000.

Site	Latitude	Sampling days	Weeks	ICES area	DNA *N*	Scales *N*
Åland Sea	60°N	29 May – 28 June	22–25	29	413	1098
Bothnian Sea	62°30′N	18 June – 6 August	25–30	30	293	507
Bothnian Bay	64°N	22 June – 4 August	26–30	31	298	529

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braska). Microsatellite genotypes were analysed using GeneImagIR™ version 3.52 fragment analysis software (Scananalytics Inc., Fairfax, Virginia).

Genetic methods

Genetic differentiation between stocks was quantified with the D_A distance of Nei et al. (1983). The neighbour-joining method (Saitou and Nei 1987) was used to construct the dendrogram. The bootstrap test over loci was performed for the neighbour-joining tree by recalculating the distance for all loci 1000 times. Distance analysis and mean heterozygosities (Nei 1973) were computed with the DISPAN package (Ota 1993). Values of F_{ST} (Weir and Cockerham 1984) and exact tests for population differentiation and Hardy–Weinberg equilibrium (Guo and Thompson 1992) were analysed using GENEPOP software (version 3.2a) (Raymond and Rousset 1995). Probabilities of Hardy–Weinberg equilibrium tests for populations were adjusted over loci using the sequential Bonferroni procedure for multiple tests (Rice 1989).

Estimation methods

Individual assignments method (program GeneClass)

The classical individual assignments method was implemented using the program GeneClass version 1.0.02 (Cornuet et al. 1999) without the simulation option. Both the baseline and mixture data were coded to provide the individual multilocus genotypes in the samples. The allele and genotype frequencies in the separate stocks were estimated by GeneClass from the baseline samples with the Rannala–Mountain formula (Rannala and Mountain 1997) under the HWLE assumptions. Therefore, none of the alleles at any locus were estimated as absent from any stock, and all genotypes were possible in each stock.

CML method (program GIRLSEM)

Mixture modeling with the CML estimation method was implemented using the program GIRLSEM (Masuda et al. 1991). The baseline data were coded to provide the total allele counts at each locus for each stock, and the mixture data were coded to provide the multilocus genotype for each individual. The allele frequencies in the separate stocks were estimated by GIRLSEM as the observed allele frequencies in the baseline samples without adjustment, and the genotype frequencies were estimated under the HWLE assumptions. The standard errors of the estimates were assessed by bootstrapping the baseline and catch samples 1000 times.

Bayesian method (program BAYES)

Mixture modeling with the Bayesian estimation method was implemented using the program BAYES (Pella and Masuda 2001). The baseline data were coded just as for GIRLSEM to provide the total allele counts at each locus for each stock, and the mixture data were coded to provide the multilocus genotype for each individual. Prior (to mixture sampling) probability distributions for allele frequencies in the separate stocks were determined by BAYES from the baseline samples. The prior average allele frequencies at a locus for each stock from BAYES were weighted averages of the corresponding observed allele frequencies and better-determined central values. The central values were simple arithmetic averages of observed allele frequencies among all baseline stocks. The weights were objectively determined by the baseline analysis described in Pella and Masuda (2001). As for the Rannala–Mountain formula used by GeneClass, baseline zeroes for allele frequencies were assumed to be sampling zeroes and were estimated to have positive values. The genotype frequencies were estimated from the allele frequencies under the HWLE assumptions.

The posterior distribution for the unknowns combines the baseline information with that of the mixture sample to estimate both the stock composition of the mixture sample and the genotypic composition of the baseline stocks. A total of 26 chains of samples were generated per application until the draws for the unknowns had stabilized and the chains had converged. Each chain corresponded to a different baseline stock with the initial proportion from that stock set at 75% and that of each of the remaining baseline stocks set at 1%. To monitor the convergence of the chains to the posterior distribution, the univariate shrink factor (Gelman and Rubin 1992) was computed for each of the baseline stock proportions. The shrink factor compares the variation within a single chain with the total variation among the chains. Depending on the iterations needed for convergence of the chains, the last 1000 or 2000 Gibbs draws of each chain were combined (26 000 or 52 000 draws) and used to describe the posterior distributions of baseline stock proportions, allele frequencies, and individual source probabilities. Averages of the posterior source probabilities for individuals, as determined from the long-run assignment frequencies by BAYES, were used to assign the individuals to their stock of origin. Each individual was assigned by the MAP rule to the baseline stock for which its average posterior source probability was the greatest.

The power of the baseline data set to resolve stock composition using these several estimation methods — classical individual assignments and mixture modeling with CML or Bayesian estimation — was assessed in two ways. First, self-assignment tests were applied in which the baseline stock samples played two roles: they were used to estimate allele frequencies at loci for the baseline stocks and to constitute mixture samples. In the role as the mixture samples, 27 samples were created from the baseline stock samples and analysed using each estimation method. The baseline stock samples, each treated as a mixture sample, constituted 26 of these self-assignment test samples, and for the last, or 27th, test sample, all 26 baseline stock samples were combined and the composite was treated as a single large mixture sample. To distinguish these test samples later, the first 26 will be called pure self-assignment test samples, as each of the test samples represented 100% of one baseline stock, and the 27th the composite self-assignment test sample. For the self-assignment tests using CML estimation, both the point estimate and bootstrap means of stock proportions over 1000 resamplings were evaluated.

Self-assignment tests are well known to be optimistic with respect to bias and precision of estimation as compared with test samples drawn independently from the baseline stocks. The mixture and baseline sampling variation of real applications is missing from self-assignment tests so that all genotypes in the test mixture are predictable from their occurrence repeated in the baseline sample. In particular, the

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Fig. 2. Genetic D_A distances (Nei et al. 1983) between the 26 Atlantic salmon (*Salmo salar*) baseline stocks of the Baltic Sea represented with a neighbour-joining tree (Saitou and Nei 1987). Distances are based on eight microsatellite loci. Country of origin (Fin., Finland; Swe., Sweden; Rus., Russia; Est., Estonia; Lat., Latvia) and sea area (designated with letters A–F; see Fig. 1) at the salmon river's mouth are indicated.

ease of identifying the source of an individual having an unusual genotype is evident for self-assignment tests unless that individual was withheld from its baseline sample. Nonetheless, the self-assignment tests provide valid comparisons of estimation methods and quantify genetic differences among the baseline stocks within their context.

Second, the two repeat baseline test samples (Neva and Tornionjoki wild) that were not troubled by the self-assignment optimism were processed by each method. The Neva test sample was expected to provide a relatively easy identification task because the stock is quite distinctive among the baseline stocks and the Tornionjoki wild sample was expected to be an especially difficult task because of the genetic similarity to its derivative hatchery stock.

Results

Genetic differentiation among baseline stocks

Genetic differentiation among the baseline stocks varied substantially. In general, the genetic distances between stocks formed a pattern (Fig. 2) similar to that observed earlier from allozyme data (Koljonen et al. 1999). Northern Baltic Sea stocks from Finland and Sweden formed one compact branch (upper 17 stocks of Fig. 2). Eastern and southern Baltic Sea stocks from Russia, Estonia, and Latvia formed another branch (lower seven stocks of Fig. 2). Two stocks from rivers in southern Sweden (Emån and Mörumsån) that drain into the southern Baltic Main Basin formed a distinct intermediate branch (between the upper 17 and lower seven stocks of Fig. 2; see Säisä et al. 2005 for more detailed analysis).

The genetic distances between the Tornionjoki wild and hatchery fish and between the Kalixälven and Tornionjoki wild fish were the same and the smallest, only 0.02. Other very small pairwise distances occurred between the Tornionjoki hatchery and Kalixälven stocks (0.03) and between two branches of the same Swedish river, Umeälven and Vindelälven (0.04). The average pairwise distance of the Tornionjoki wild stock from all other baseline stocks was 0.18 and that of the Neva stock was 0.21.

The overall F_{ST} for all baseline stocks varied among loci from 0.06 (*Ssa197*) to 0.16 (*Ssa171*) and was on average 0.11 (Table 3). The F_{ST} for all baseline stock pairs varied from the maximum of 0.29 between salmon from the Estonian Kunda River and Finnish Iijoki to the minimum of 0.003, which was measured between the Tornionjoki wild and hatchery derivatives and also the Tornionjoki wild and Kalixälven stocks. Other F_{ST} values smaller than 0.01 occurred between stocks from Umeälven and Vindelälven (0.006) and between stocks from Emån and Mörrumsån (0.005). Small pairwise F_{ST} values might indicate difficulties in distinguishing these stocks from each other.

Genetic differentiation among baseline stock pairs was highly significant ($P < 0.001$) in all other cases, but not be-

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Table 3. The eight DNA microsatellite loci sampled from 26 Atlantic salmon (*Salmo salar*) stocks within the Baltic Sea and their F_{ST} values (Weir and Cockerham 1984), mean heterozygosities (H_T), and number of alleles per locus (N) in the baseline data.

Locus	F_{ST}	H_T	N
Ssa85	0.079	0.85	19
Ssa171	0.161	0.67	23
Ssa197	0.058	0.90	21
Ssa202	0.096	0.77	9
Ssa289	0.156	0.75	8
SSOSL85	0.093	0.74	14
SSOSL417	0.139	0.89	19
SSOSL438	0.103	0.65	12
All	0.109	0.78	125

tween Vindelälven and Umeälven ($P < 0.05$) and between Emån and Mörrumsån ($P < 0.05$). The average total heterozygosity for all loci was 0.78 (0.65–0.90) (Table 3) and the average over populations was 0.69 (0.59 for Kunda to 0.76 for Ljungan). The number of alleles varied from 8 to 23 among loci (Table 3). According to GENEPOP analysis, deviations from Hardy–Weinberg equilibrium, at least in one locus and with a probability smaller or equal to 0.01, were observed in 8 of 26 populations (Tornionjoki hatchery, Kalixälven, Skellefteälven, Umeälven, Ångermanälven, Ljungan, Dalälven, and Pärnu) and in 14 cases out of 208 tests. When Bonferroni correction for the tested number of loci was applied for populations, deviations smaller or equal to 0.01 were observed in three hatchery populations (Tornionjoki hatchery, Skellefteälven, and Dalälven) and in one wild population (Ljungan). A more detailed analysis of the microsatellite data is provided in Säisä et al. (2005).

Self-assignment tests for the baseline

Clear differences were seen in the resolving power from the self-assignment tests between the classical individual assignments and the mixture modeling methods (Table 4). The percentage of individuals correctly assigned to their source by the classical individual assignments method using GeneClass for the pure self-assignment test samples ranged from only 44% (for the Tornionjoki hatchery stock) to 94% (for the Lögde stock), with an average of 75% correct per stock. For the composite self-assignment test sample, 71.5% were correctly assigned (1221 correctly assigned to their sources of 1708 fish). The classical individual assignments method performs identically on pure and composite self-assignment test samples because each genotype is assigned to the same stock regardless of the assignments of other fish in the mixture sample. However, if the composite self-assignment test was done by a "leave-one-out" procedure, in which the particular fishes were withheld from their population baseline sample when performing their assignment, the percentage of correct assignment was only 55.8% (953 correctly assigned from 1708 fish).

In contrast, when mixture modeling with the CML estimation method was applied to the pure self-assignment test samples and the point estimate was used, the estimated proportion from the correct baseline stock was lowest at 96.5%

Table 4. Means and ranges of correct percentages for the 26 pure self-assignment tests with the Atlantic salmon (*Salmo salar*) baseline samples by estimation method.

	Correct stock (%)	
Estimation method	Mean	Range
Classical individual assignment		
Assignments (GeneClass) direct	75.1	44–94
Assignments (GeneClass) leave-one-out	55.8	—
Mixture modeling		
CML method (GIRLSEM) direct	98.7	96.5–100.0
CML method (GIRLSEM) bootstrap	87.1	76.7–96.5
Bayesian method (BAYES)	97.0	91.0–98.7
Individual assignments (BAYES)	99.9	98.0–100.0

(for the Gauja stock) and averaged 98.7% (96.5%–100%) over all of the baseline stocks (Table 4). The largest error in the CML point estimate was thus –3.5% and the average error over all baseline stocks was –1.3%. When baseline and mixture sampling variances were included by bootstrapping, the mean estimated proportion from the correct baseline stock still averaged 87.1% (76.7% for Umeälven to 96.5% for Neva) over the baseline stocks. Any major discrepancy exceeding 20% error in mean estimated proportions was restricted to each of the three most similar (pairwise F_{ST} less than 0.01) stock pairs (Tornionjoki wild – Tornionjoki hatchery, Umeälven–Vindelälven, and Emån–Mörrumsån).

In the large composite self-assignment test sample, the actual stock proportions were more equal and small (1.5%–9.9%) (Table 1). When the CML estimation method was applied to the composite self-assignment test sample, all the stocks were detected as present in the mixture; that is, confidence intervals about the bootstrap means did not include zero. The smallest true proportion was for the Pärnu salmon stock, only 1.5% of the total baseline data, with the baseline sample size of 26 fish, and the bootstrap mean estimate was very precise at 1.4% (±0.4), an error of only –0.1%. Among stocks, the largest error of the CML point estimate was 2.1% for the Kalixälven stock (9.9% true proportion), which was clearly higher than the next largest error of 1.0% for the Tornionjoki wild stock (6.9% true proportion), and the absolute error averaged 0.4% over all baseline stocks (baseline average of true proportions was 3.8%). So the combination of more equal true stock proportions and the increase of mixture sample size from the mean of about 66 in the pure self-assignment tests to about 1700 in the composite test clearly increased accuracy and precision.

When the Bayesian estimation method replaced the CML method in mixture modeling for the pure self-assignment tests, the estimated correct proportion was at the same high level with an average of 97% correct over all baseline stocks if the mean of the posterior distribution was used as the point estimate (Table 4). The average error was –3%, and the largest, and only error over 5% in absolute value, was –8.1% for the Tornionjoki hatchery stock. Thus, the downward error within the three most similar stock pairs was clearly less when estimated with the Bayesian method (–8.1% for Tornionjoki wild – Tornionjoki hatchery, –4.5% for Umeälven–Vindelälven, and –3.8% for Emån–Mörrumsån)

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Table 5. Estimated stock proportions of the repeat baseline test sample (n = 50) consisting entirely of Atlantic salmon (*Salmo salar*) from Neva by the classical individual assignments method (program GeneClass), mixture modeling with the conditional maximum likelihood (CML) method (program GIRLSEM) or the Bayesian method (program BAYES), and individual assignments using the maximum average posterior source probability from BAYES.

	Individual assignments	Mixture modeling				
		CML method (GIRLSEM)		Bayesian method (BAYES)		Individual assignments (BAYES)
	GeneClass					
Stock	% of individuals	Proportion (%)	SE	Proportion (%)	SD	% of individuals
Tornionjoki wild	2.0	4.4	4.4	0.1	0.4	0.0
Luleälven	0.0	4.5	4.5	0.1	0.4	0.0
Byskeälven	2.0	8.3	6.9	0.1	0.4	0.0
Indalsälven	2.0	0.0	0.0	0.1	0.4	0.0
Emån	0.0	2.4	1.9	0.1	0.4	0.0
Mörrumsån	0.0	2.2	1.7	0.1	0.4	0.0
Neva	88.0	73.9	8.4	97.1	2.4	100.0
Kunda	2.0	0.0	0.0	0.1	0.3	0.0
Keila	0.0	2.2	2.3	0.1	0.5	0.0
Pärnu	2.0	0.0	1.0	0.1	0.4	0.0
Gauja	0.0	0.0	1.3	0.4	1.1	0.0
Daugava	0.0	0.0	1.9	0.2	0.7	0.0
Venta	2.0	2.2	2.4	0.7	1.4	0.0
Total	100.0	100.0		100.0		100.0

Note: SE estimated from the bootstrapped distribution for the CML estimate and SD from the posterior probability distribution for the Bayesian estimate are given. With BAYES, 1000 Markov chain Monte Carlo samples were drawn from each of 26 chains after convergence and averages are shown. Only stocks into which fish were assigned are listed.

than with the CML method for which these downward errors exceeded 20% for those stock pairs.

When mixture modeling with the Bayesian estimation method was applied to the composite self-assignment test sample, similarly as with CML estimates, all of the stocks were detected as present in the mixture considering that all of the lower boundaries of the 95% posterior intervals for the stock proportions were greater than zero. Among stocks, the error, or the difference between the posterior average stock proportion and the actual proportion, was at most +1.4% (for 9.9% true Kalixälven proportion) and absolute error averaged 0.3%. The greatest tendency for underestimation was seen in the Tornionjoki hatchery (–0.8% error for 7% true proportion) and Luleälven (–0.7% for 3.5% true proportion) stocks, which both obviously included fewer different multilocus genotypes than the others.

When the individuals of the pure self-assignment test samples were assigned by the MAP rule to the source with the largest average posterior source probability, almost all were correctly assigned (Table 4). Only 2 out of 1708 fishes were incorrectly assigned, one from the Tornionjoki hatchery stock and one from Emån stock. The maximum average posterior source probabilities were generally over 0.98, and the proportion of incorrect individual assignment was at most 2.9% (for the Emån stock). Thus, as expected, individual assignments from average posterior source probabilities computed by BAYES resulted in superior source identification compared with that by the classical individual assignments method.

Repeat baseline test samples

When the independent, repeat baseline test samples were used for comparing methods, measures of accuracy and precision included baseline sampling variance and temporal changes in allele frequencies within the stocks. Importantly, novel alleles can be drawn in the test samples from the stocks that earlier had sampling zeroes in their place in the original baseline samples. The methods showed different resolution powers for the two samples. The Neva stock was relatively easy to identify in the Neva test sample, and 88%, or 44 of the 50 individuals, were correctly assigned even with the classical individual assignments method (GeneClass) (Table 5). The CML method (GIRLSEM) performed the least well with a point estimate of 73.9% from the Neva stock. The Bayesian method (BAYES) had the highest power, as the mean of the posterior distribution for stock composition was 97.1% from the Neva stock. Each of the 50 fishes was assigned correctly by the BAYES MAP rule to the Neva stock, and nearly all were assigned with high certainty: 48 individuals had nearly 100% posterior source probability for the Neva stock, one fish had 99%, and only one fish had 48%.

Application of the three methods to the repeat wild Tornionjoki test sample revealed that the classical individual assignments method (GeneClass) could not distinguish wild from the hatchery derivative well. A large proportion (23.2%, or 13 of 56 fishes) of the wild Tornionjoki repeat test sample was misassigned to the hatchery stock (Table 6). In all, only half of the wild Tornionjoki test fish (50.0%, or 28 of 56 fishes) were assigned to either the wild or hatchery Tornionjoki stock. A large contribution (25.0%, or 14 fishes) was erroneously assigned to the very similar Kalixälven stock.

With mixture modeling using the CML estimation method, the estimated proportion from either the wild or the hatchery Tornionjoki stock increased to 68.9%. However, the error towards the Kalixälven stock remained at about the same level

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Table 6. Estimated stock proportions of the repeat baseline test sample ($n = 56$) consisting entirely of Tornionjoki wild Atlantic salmon (*Salmo salar*) by the classical individual assignments method (program GeneClass), mixture modeling with the conditional maximum likelihood (CML) method (program GIRLSEM) or Bayesian method (program BAYES), and individual assignments using the maximum average posterior source probability from BAYES.

	Individual assignments	Mixture modeling				
	GeneClass	CML method (GIRLSEM)		Bayesian method (BAYES)		Individual assignments (BAYES)
Stock	% of individuals	Proportion (%)	SE	Proportion (%)	SD	% of individuals
Tornionjoki wild	26.8	54.3	15.8	91.0	4.0	98.2
Tornionjoki hatchery	23.2	14.6	10.2	1.3	10.5	0.0
(Tornionjoki total)	(50.0)	(68.9)		(92.3)		(98.2)
Simojoki	7.1	3.1	2.9	0.2	0.9	0.0
Iijoki	5.4	0.0	2.4	0.2	1.0	0.0
Oulujoki	1.8	0.0	0.3	0.6	1.4	0.0
Kalixälven	25.0	24.2	14.0	3.6	8.9	0.0
Luleälven	3.6	0.0	0.3	0.1	0.4	0.0
Byske	1.8	0.0	2.3	0.2	0.8	0.0
Skellefteälven	3.6	0.0	0.6	0.7	1.6	0.0
Lögde	1.8	1.9	1.9	0.9	1.5	1.8
Emån	0.0	1.9	1.8	0.0	0.3	0.0
Ångermanälven	0.0	0.0	0.0	0.2	0.7	0.0
Total	100.0	100.0		~100.0		100.0

Note: SE estimated from the bootstrapped distribution for the CML estimate and standard deviation SD from the posterior probability distribution for the Bayesian estimate are given. With BAYES, 2000 Markov chain Monte Carlo samples were drawn from each of 26 chains after convergence and averages are shown. Only stocks in which the proportion was higher than 0.1% are listed.

(24.2%). Mixture modeling with the Bayesian estimation method resulted in an estimated proportion of 91.0% from the correct Tornionjoki wild stock and only 1.3% from the incorrect Tornionjoki hatchery stock. The error towards the Kalixälven stock was low at 3.6%. Assignment of individuals by the MAP rule with the Bayesian estimation method resulted in only one error (one incorrectly assigned of 56 fish). For all but two individuals, the maximum average posterior source probability was higher than 0.86 (for the two exceptions, it was 0.46 and 0.73) and for 40 (71%) of the individuals higher than 0.95 (average posterior source probability was at least 0.95). Thus, the posterior source probabilities for individuals seemed remarkably reliable for determining their sources.

Stock proportions of actual catches in the northern Baltic Sea

Catches were sampled within the northern Baltic Sea at three sites (proceeding northward): the Åland Sea, Bothnian Sea, and Bothnian Bay (Fig. 1). For management purposes, five stock groups were defined according to the location of their home river mouths in three sea areas — Gulf of Bothnia, Gulf of Finland, and Baltic Main Basin — and source of production within the Gulf of Bothnia — wild, Finnish hatchery, or Swedish hatchery (Table 7). In all three samples, the sum of estimated proportions of eastern and southern stocks from the Gulf of Finland and the Baltic Main Basin was minor (<1%). The estimated proportion of wild stocks from the Gulf of Bothnia was quite stable among sampling locations and varied from 36% to 43%, with the lowest value in the Bothnian Sea catch (Fig. 3). The total proportions from hatcheries of the Gulf of Bothnia also tended to be stable, ranging from 56% to 64% among locations, but the magnitudes of the Finnish and Swedish hatchery components changed opposite to each other among samples (Fig. 3). In the southern sample from near the Swedish coast in the Åland Sea, roughly 40% of fish were estimated from Swedish hatchery fish. The proportion of Swedish hatchery fish, however, decreased in the more northerly samples, being about 20% in the Bothnian Sea and only about 10% in Bothnian Bay. On the other hand, the proportion of Finnish hatchery fish was about 15% in the southern sample, 45% in the Bothnian Sea sample, and 49% in the Bothnian Bay sample.

When catches are known and representative genetic samples for stock composition estimation are available, estimates of numbers of fishes caught per stock can be obtained. According to available catch statistics, the total number of Atlantic salmon caught in 2000 at Åland Sea (part of International Council for the Exploration of the Sea statistical area 29) by Finns was 10 512 fish, which weighed a total of 74 000 kg. If these numbers are assumed to be known without error, the total number of wild fish caught in the Åland Sea fishery in 2000 was around 4500 (95% probability interval = 3800–5200) fish weighing about 32 metric tons (95% probability interval = 27–37 metric tons) (Table 8). Corresponding catches in 2000 by Finns in the whole Gulf of Bothnia (including the Åland Sea, Bothnian Sea, and Bothnian Bay) was 42 786 fish weighing 206 metric tons (International Council for the Exploration of the Sea statistical area 29 had 12 275 fish, area 30 had 8746 fish, and area 31 had 21 765 fish). The proportion of wild salmon stocks in Finnish catches in the Gulf of Bothnian area for the whole year 2000 was 39% (95% probability interval = 31%–48%) when estimated by the catch weighted average of wild salmon proportions at the three sample locations. An esti-

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Table 7. Stock group proportions in Finnish Atlantic salmon (*Salmo salar*) catch samples in 2000 on the basis of genetic variation at eight microsatellite loci.

Catch site	Group[a]	Mean	SD	2.5%	Median	97.5%
Åland Sea (60°N)	Gulf of Bothnia					
	Wild	43.3	3.4	36.6	43.3	49.9
	Finnish hatchery	14.6	2.9	9.3	14.4	20.7
	Swedish hatchery	41.2	3.1	35.3	41.2	47.3
	Gulf of Finland	0.1	0.1	0.0	0.0	0.5
	Main Basin	0.8	0.5	0.2	0.8	2.0
	Total	100.0			99.7	
Bothnian Sea (62°30′N)	Gulf of Bothnia					
	Wild	35.7	5.8	24.8	35.6	47.2
	Finnish hatchery	44.6	5.8	33.3	44.7	55.9
	Swedish hatchery	19.2	3.1	13.5	19.1	25.6
	Gulf of Finland	0.0	0.1	0.0	0.0	0.4
	Main Basin	0.5	0.4	0.0	0.4	1.6
	Total	100.0			99.7	
Bothnian Bay (64°N)	Gulf of Bothnia					
	Wild	39.5	5.2	29.7	39.3	49.8
	Finnish hatchery	49.3	5.5	38.2	49.4	59.6
	Swedish hatchery	10.8	2.7	5.9	10.6	16.6
	Gulf of Finland	0.3	0.5	0.0	0.1	1.6
	Main Basin	0.2	0.3	0.0	0.0	1.0
	Total	100.0			99.4	

Note: Estimates are the sums of posterior average stock proportions computed by mixture modeling and the Bayesian estimation method using the program BAYES (Pella and Masuda 2001).

[a]Stocks included in the groups are as follows: (*i*) Gulf of Bothnia wild: Tornionjoki wild, Simojoki, Kalixälven, Byske, Vindelälven, Lögde, and Ljungan. (*ii*) Gulf of Bothnia Finnish hatchery: Tornionjoki hatchery, Iijoki, and Oulujoki. (*iii*) Gulf of Bothnia Swedish hatchery: Luleälven, Skellefteälven, Umeälven, Ångermanälven, Indalsälven, Ljusnan, and Dalälven. (*iv*) Gulf of Finland: Neva, Kunda, and Kesla. (*v*) Baltic Main Basin: Emån, Mörrumsån, Pärnu, Gauja, Daugava, and Venta.

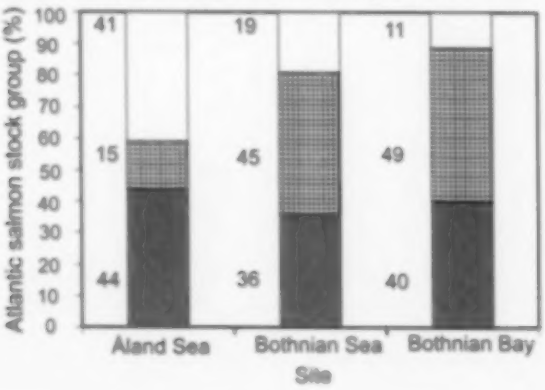

Fig. 3. Estimated proportions of Atlantic salmon (*Salmo salar*) stock groups in Finnish catches sampled in 2000 from three sites in the northern Baltic Sea based on variation at eight DNA microsatellite loci. Estimates are the average posterior stock proportions computed by mixture modeling and the Bayesian estimation method using the program BAYES (Pella and Masuda 2001). Shaded bars, wild stocks; crosshatched bars, Finnish hatchery stocks; open bars, Swedish hatchery stocks.

mate of 16 800 (95% probability interval = 13 100 – 20 300) wild fish for the whole Gulf of Bothnia area in the Finnish commercial fishery, weighing about 81 metric tons (95% probability interval = 63–98 metric tons), results if wild and hatchery fish had the same size.

Discussion

Method comparison

Uncertainty in individual assignments and stock composition estimation arises from at least six sources. Imperfect discrimination power results from (*i*) the finite existing differentiation among baseline stocks, which is limited by the overlap among the stocks' multilocus genotype distributions for the available loci. Although the existing differentiation is presumably the most that is technologically available and economically affordable, it can be wasted by use of (*ii*) inefficient estimation methods. Additional sources of uncertainty include (*iii*) sampling variation from the baseline stocks and the mixture, (*iv*) temporal changes in the true genetic composition of the baseline stocks between the times of baseline and mixture sampling, (*v*) violation of modeling assumptions, such as HWLE, and (*vi*) incomplete baselines with some mixture individuals contributed by out-populations (see Smouse et al. 1990).

The discrimination power of the particular baseline data and the relative efficiencies of the estimation methods can be assessed by self-assignment tests, although the results do not include the uncertainty caused by sampling variance or temporal variation of baseline allele frequencies. Demonstrated discrimination power by self-assignment tests is a necessary

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Table 8. Composition of Finnish Atlantic salmon (*Salmo salar*) catches in the Åland Sea during 2000 by five stock groups as estimated by the Bayesian method (posterior average) using the program BAYES and estimated number and kilograms of fish for the apportioned total catch and their 95% probability intervals when numbers and kilograms were assumed to be known without error.

Origin of stock group	Proportion (%)	Catch (number)	95% probability interval	Catch (kg)	95% probability interval
Gulf of Bothnia					
Wild	43.3	4 552	3847–5243	32 045	27 087–36 915
Finnish hatchery	14.6	1 533	981–2175	10 790	6 905–15 312
Swedish hatchery	41.2	4 334	3707–4973	30 513	26 095–35 013
Gulf of Finland	0.1	5	0–48	37	0–340
Baltic Main Basin	0.8	88	16–212	622	562–1495
Total	100.0	10 512		74 008	

but not a sufficient condition for successful stock proportion estimation or individual source identification. Uncertainty in baseline allele frequencies can downgrade performance in an actual application from that of the self-assignment tests. Violation of model assumptions is to some extent included in the self-assignment tests depending on the analytical methods used. In the present study, each method and its implementing program estimated the baseline genotype frequencies of the observed mixture multilocus genotypes under the HWLE assumptions. These assumptions have been tested commonly for fish populations and violations are rarely detected. Here, deviations in some populations were observed. In hatchery populations, temporary deviations may occur as a result of hatchery practises. However, even if the assumptions were not fully met, they provide approximations to the multilocus genotype frequencies that the populations will reach under random mating and that would be more difficult to obtain otherwise. Analysis of repeat baseline test samples reveals if the modeling assumptions are robust enough for practical use.

In contrast with self-assignment tests, repeat baseline test samples always include sampling variance and can include violations of modeling assumptions. Further, if the test and baseline samples are taken in different years, temporal variation in baseline allele frequencies may also be included. Here, for the repeat test samples, 2 years (Neva, 1995–1997) to 7 years (wild Tornionjoki, 1993–2000) passed between test and later baseline sampling, and in addition, the entire baseline was collected over several years from 1993 to 2002. Some temporal variation in the genetic composition of the baseline stocks probably occurred during the interval.

Self-assignment

Self-assignment tests showed that the classical individual assignments method using the eight DNA microsatellite loci available (average F_{ST} equal to 0.11) did not discriminate well among genetically similar Atlantic salmon stocks within the Baltic Sea. The percentage of correct assignments was only 71.5% for the composite of all baseline individuals, even with the same multilocus genotypes of individual fish serving as both baseline and unknown samples and only 55.8% correct with the leave-one-out procedure. Manel et al. (2002) found that using 10 microsatellite loci, nearly all individuals could be assigned with GeneClass with high statistical certainty when only two highly differentiated populations (F_{ST} = 0.15–0.20) were mixed in equal proportions. In the case of Neva salmon, the mean F_{ST} over all baseline stocks was 0.1 and none was below 0.05, whereas for the wild Tornionjoki stock, seven of the pairwise F_{ST} values were under 0.05. Cornuet et al. (1999) reported that with 10 simulated population mixtures of equal proportions composed of 30 individuals per population, 10 loci, and F_{ST} = 0.1, the correct assignment rate using GeneClass was close to 100%. Therefore, classical individual assignments seem to work relatively well if the F_{ST} values exceed 0.1 and the mixture proportions are equal, but greater error may easily occur if the separation between stocks is less than that and particularly if the mixture proportions are unequal with the most similar stocks in greatest abundance. The F_{ST} for Atlantic salmon stocks of the same geographic area is often less than 0.1. Here, about half of the 325 stock pairs (49%) had pairwise F_{ST} values less than 0.1, one-fifth (19%) of them less than 0.05, and five less than 0.01. As F_{ST} estimation does not, however, take into account the identity of alleles, but only differences in frequencies, the F_{ST} estimates are the same, regardless of whether the sets of alleles overlap or not. Therefore, the separation might actually be higher than that indicated by the F_{ST} value. If cost is not a concern, discrimination power can be increased by increasing the number of loci used for the assignments, but the overlapping of multilocus genotype distributions among baseline populations is ultimately limiting. With the given set of loci, discrimination power can only be increased by use of more efficient statistical procedures.

According to the self-assignment tests, the discrimination power was good and the biases in general were reduced when mixture modeling was used instead of the classical individual assignments method. For the point estimates, the accuracy was even slightly better in the pure self-assignment tests for the CML method than for the Bayesian method with averages correct equal to 98.7% and 97.0%, respectively. This higher performance is understandable for a test in which the baseline allele frequencies were actually known without error as is assumed by the CML method. Perfect baseline information is, however, not available for routine estimation when mixture and baseline sample sizes are limited and may have been obtained in different years. When sampling variance was included with bootstrapping, the CML average correct for the pure self-assignment test declined slightly to 87.9% among stocks. However, resolution

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by the CML method for the three most similar stock pairs was less than for the Bayesian method: maximum downward errors were over 20% by the CML method compared with only 8.1% for the Bayesian method. Therefore, mixture modeling with the Bayesian method improved accuracy especially for difficult cases of similar stocks.

In the self-assignment tests, individual assignments by the MAP rule and mixture modeling with the Bayesian estimation method were more accurate than by the classical individual assignments method. With pure self-assignment tests, the performance of the method was seen to vary among baseline stocks depending on baseline sample sizes and the degree of similarity (overlap of multilocus genotype distributions) with other stocks. Some tendencies for either under- or overestimation could be observed. This information can be used in planning stock groupings for actual fisheries analysis. For example, pooling estimates of geographically close stock pairs would retain much of the practical value of the estimation while reducing bias and improving precision.

Repeat baseline test samples

When the uncertainty related to sampling was included by the repeat baseline test samples, comparative performances by methods differed materially between the two test samples. For the relatively distinct Neva salmon stock, the classical individual assignments method performed better than mixture modeling with the CML method. Almost certainly, the better performance by the classical individual assignments method was mainly due to its correction of sampling zeroes with the Rannala–Mountain formula, whereas no correction was attempted by the CML method. Not all of the same alleles occurred in the Neva 1995 test sample and 1997 baseline sample. However, the allele frequencies of Neva salmon deviated sufficiently from those of the other baseline stocks to allow accurate individual assignments when sampling zeroes were corrected.

The wild Tornionjoki test sample provided a greater challenge for which differences among methods in their performances increased. Correct stock proportion estimation increased from 50% by the classical individual assignments method to 69% for mixture modeling and the CML method and finally to 92% by mixture modeling and the Bayesian method. The roughly 20% improvement in correct estimated stock proportion by the CML method from the classical individual assignments method resulted from the utilization of mixture modeling. The approximately 20% improvement in the correct estimation from the CML method to the Bayesian method resulted from better handling of baseline sampling zeroes and the incorporation of mixture sample information about allele frequencies in the contributing stocks with that from the baseline samples.

The sampling variance caused by mixture and baseline sampling can be estimated by bootstrapping in the CML method, which is a realistic method to assess the variability resulting in the composition estimates. Bootstrapping can be used to make confidence intervals for the unknowns. In the Bayesian method, the generation of large numbers of samples from the posterior distribution of the stock proportions and allele frequencies replaces the bootstrapping of the CML method. Samples from the posterior distribution can be used to construct probability intervals for the unknowns, which are the replacement for confidence intervals. In contrast with the indirect and commonly misinterpreted confidence intervals, these probability intervals allow direct probability statements for the unknowns that are easily understood.

The baseline samples were sufficient for quite accurate stock proportion estimation of the repeat wild Tornionjoki test sample using mixture modeling with the Bayesian method. The Tornionjoki wild stock is a relatively large stock, so marked drift of allele frequencies would be unexpected. On the other hand, population substructure in large rivers and organization of representative sampling of all tributaries might cause problems. Temporal changes in baseline allele frequencies should also be taken into account as a potential source of error. In general, however, any effects of changes caused by genetic drift can be compensated for by collecting baseline data over several years (Waples 1990). The importance of repeated sampling depends on the life history of the species concerned and on the degree of concurrence of the year-classes. Atlantic salmon has overlapping generations, and partly for that reason, the temporal variation of allele frequencies in large natural stocks may well be negligible, whereas in small natural stocks or in hatchery stocks, genetic drift can cause more pronounced changes. For the Atlantic salmon populations, allele frequencies were very stable over a 50-year period in a large wild stock (F_{ST} = 0.007) but changed more for a hatchery stock over 30-year period (Iijoki from 1962 to 1995, F_{ST} = 0.029) (Säisä et al. 2003). Here, the time interval between the wild Tornionjoki samples was 7 years (1993–2000), and their estimation accuracy was still very good. One of the advantages of the Bayesian method is that it is able to update the baseline allele frequencies on the basis of information contained in the annually cumulating mixture samples. However, this updating from the mixture samples may not completely replace the need of occasionally resampling directly from the baseline stocks.

Incompleteness of baseline data did not apply in test sample analysis, but for the true catches, it might be a source of uncertainty. Here, the baseline data covered well the smolt production of the rivers, especially for the northern Baltic Sea where the catches were made. For the hatchery stocks, very nearly 100% of the production was covered. For the wild stocks, 95% of the total wild smolt production was covered when estimated from the 1998 production level, which was about 400 000 wild smolts for the Gulf of Bothnia area (Anonymous 2004). However, six small Swedish wild stocks exist (combined output of about 20 000 smolts per annum) for which no baseline stock samples were yet available. Their contribution in the total catch is, however, considered negligible.

Use of the GIRLSEM, SPAM (Debevec et al. 2000), or BAYES mixture modeling program is strictly applicable only when all of the mixture individuals are believed to derive from the populations included in the baseline samples. When out-populations occur in the mixture, two tracks have been taken by the methods of individual assignments and mixture modeling. In the method of individual assignments, each mixture genotype is considered in isolation of the others, and those genotypes that are relatively infrequent among the baseline stocks (Cornuet et al. 1999) are identified as be-

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ing from out-populations. Out-population genotypes with greater frequencies among baseline stocks cannot be detected by this method. On the other hand, mixture modeling using estimation methods different from those so far considered brings the entire mixture sample to bear on the question and identifies out-population individuals through the Wahlund effect and the HWLE condition. Smouse et al. (1990) first proposed sequential testing for populations present in the mixture sample and composition estimation for populations detected, but the problem has recently been attacked with modern computational methods.

The recent mixture modeling of Pritchard et al. (2000) and Dawson and Belkhir (2001) comprises Markov chain Monte Carlo methods that can stochastically partition the mixture genotypes into HWLE subsets even if no baseline samples are available. Even out-population genotypes with high frequencies among baseline stocks could be detected by their membership in HWLE subsets from out-populations. The computer program STRUCTURE is based on the method of Pritchard et al. (2000) and identifies the HWLE subsets to their baseline and out-population sources. STRUCTURE can also accommodate situations like the present study in which baselines are essentially complete and no out-populations occur. Information about population composition that is provided by the HWLE condition among mixture individuals is not extracted by the mixture modeling of GIRLSEM, SPAM, or BAYES and its use might offer some increase in the separation power when applied together with known baseline data and mixture modeling. Regardless, the point to be emphasized here is that accuracy and precision of stock composition estimation are enhanced by use of mixture modeling as compared with classical individual assignments. This conclusion differs from that derived in a related study discussed next.

Potvin and Bernatchez (2001) concluded from a comparison of the classical individual assignments method using the assignment test of Paetkau et al. (1995) versus mixture model analysis via SPAM that the classical individual assignments method was more appropriate for analyses of mixtures of landlocked Atlantic salmon in Lake St-Jean, Quebec. Their conclusion that the classical individual assignments method was to be preferred had three bases, two of which are questionable and the last neutral for the methods. First, the classical individual assignments method was applied to a four-population composite of the unlabeled baseline individuals and a simulated mixture of the same populations. The classical individual assignments method was quite accurate for individuals of both mixtures, the first of which was composed of approximately equal proportions of baseline individuals and the second composed of exactly equal proportions by design of the simulation. Evidently, as was true for Cornuet et al. (1999) and Manel et al. (2002), the authors were not aware that the classical individual assignment rule is the optimal Bayes' classifier from decision theory (e.g., Pella and Masuda 2005) for mixtures of equal population proportions and had inadvertently created an instance favourable to the method. When the authors tested the mixture model for estimating the compositions, they considered the same baseline composite of unlabeled baseline individuals but changed the simulated mixture to have quite uneven population proportions. The mixture model performed well in both cases. No evaluation was performed by the classical individual assignments method applied to the uneven simulated mixture, but had it been done, the method may well have suffered in the comparison. The second basis for preferring the classical individual assignments method was their misapprehension of the apparent precision of composition estimates for the unknown mixtures from individual assignments and mixture modeling. The confidence intervals constructed for the population proportions from individual assignments were narrower (and so preferable from the authors' viewpoint) than those from mixture modeling because the uncertainty in the sources of the individuals was inadvertently omitted for the classical individual assignments method but was correctly included in mixture modeling. The uncertainty in the individual assignments should have been included as described for classification-based composition estimation by Millar (1987) or Wood et al. (1987) (also see Pella and Masuda 2005). The third basis for their conclusion was that happily, the estimates from the two methods were highly correlated in applications to unknown mixtures, so choice of method was not critical in their study.

Advantages of Bayesian estimation

The methodology that underlies the program BAYES has several features responsible for its superior performance. First, the baseline allele frequencies are estimated by a probability distribution whose mean is a weighted average of the observed frequencies and a more stable central value for the entire set of baseline samples. Second, the probability distribution for the allele frequencies derived from the baseline samples is updated to include information from the mixture sample. When baseline samples from some stocks are small, and the number of mixture sample individuals from such stocks is meaningful, the addition of the mixture sample information can be valuable to better estimate the allele frequencies of baseline stocks and thereby improve the stock composition estimates. Although both the Bayesian and CML methods use mixture modeling, these two features largely explain the advantage that the BAYES program had when compared with GIRLSEM. The sampling zeroes of alleles, which do not occur in self-assignment tests, caused a reduction in performance when GIRLSEM was applied to the repeat baseline test samples. The CML method is also used in the program SPAM (Debevec et al. 2000), but that program now includes the option of correcting sampling zeroes from the baseline samples. However, SPAM does not use the mixture sample information to update allele frequencies from the baseline samples. The advantage of individual assignments by BAYES over GeneClass is due to its use of estimated stock proportions as prior source probabilities for the individuals. The assignment rule in GeneClass, which attributes each individual to the baseline stock in which its genotype is most frequent, results when equal prior stock proportions are used in place of those estimated by mixture modeling. When such an assignment rule is used on a mixture with roughly equal proportions from the baseline stocks, it will perform well, but when the source proportions are very uneven, as was the case for the two repeat baseline test samples, the rule may perform poorly as was seen here. When the mixture sample contains little information about the stock proportions, the estimated stock proportions will

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be imprecise and choice of method is unimportant. The tendency to evaluate performance of various assignment methods on mixtures with equal proportions is natural but needs to be countered by including mixtures with uneven proportions as well.

Analysis of the actual catch samples

For the management point of view, five important groups could be satisfactorily separated in the three actual catch samples. In the Gulf of Bothnia catch area, practically none of the fish were from the Gulf of Finland or from the Baltic Main Basin. However, each of the three Gulf of Bothnia groups comprised substantial proportions of the catches (10.8%–49.3%) at each of the three sampling locations in the Åland Sea, Bothnian Sea, and Bothnian Bay, and their spatial distributions differed. The proportion of Finnish hatchery stocks increased, and that of Swedish hatchery stocks decreased, going northward. On the other hand, Gulf of Bothnia wild stocks had a more even distribution among locations with an estimated 43%, 36%, and 40% of the total Finnish Atlantic salmon catches going northward and composed an estimated 39% (95% probability interval = 31%–48%) of the overall Finnish catch in the Gulf of Bothnia during 2000. The corresponding 95% probability intervals at the three sampling locations were roughly symmetric about these point estimates, or posterior means, at ±6.7%, ±11.7%, and ±10% from sample sizes of about 400, 300, and 300, respectively. Generally speaking, a maximum width of ±10% probability interval could be achieved for the critical wild group in the future with a sample of about 300 fish collected from catches of a fishing area.

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Can growth be used as a surrogate measure of walleye (*Sander vitreus*) abundance change?

Greg G. Sass and James F. Kitchell

Abstract: We examined several models to determine the relative contributions of density-dependent and density-independent factors on walleye (*Sander vitreus*) growth in the ceded territory of Wisconsin from 1990 to 1999. We then used independent data from 2000 to determine how well each model predicted walleye growth and examined several models to determine if growth could be used to predict density. Adult density best predicted the mean size of age-3 males ($\bar{L}_{3m}$) and age-5 females ($\bar{L}_{5f}$). Density-independent measures of pH, the morphoedaphic index, maximum depth, and conductance had a positive influence on growth, while adult density negatively affected growth. The solely density-dependent models predicted $\bar{L}_{3m}$ and $\bar{L}_{5f}$ poorly in 2000. The addition of density-independent factors improved model predictions of $\bar{L}_{3m}$ and $\bar{L}_{5f}$. Walleye growth did not predict adult walleye densities. Regional patterns in walleye growth were correlated with surrogate measures of lake productivity and to a lesser extent adult density. Density dependence had a relatively weak affect on growth patterns, suggesting that growth cannot be used as a surrogate method for monitoring abundance change.

Résumé : Nous avons utilisé plusieurs modèles afin de déterminer les contributions relatives des facteurs liés à la densité et des facteurs indépendants de la densité sur la croissance du doré (*Sander vitreus*) dans le territoire concédé du Wisconsin de 1990 à 1999. Les données indépendantes de 2000 nous ont ensuite servi à vérifier dans quelle mesure chaque modèle a pu prédire la croissance des dorés. Nous avons aussi analysé plusieurs modèles pour déterminer si la croissance pouvait servir à prédire la densité. La densité des adultes permet de prédire le mieux la taille moyenne des mâles d'âge trois ($\bar{L}_{3m}$) et des femelles d'âge cinq ($\bar{L}_{5f}$). Les valeurs du pH, de l'indice morphoédaphique, de la profondeur maximale et de la conductance, toutes indépendantes de la densité, ont un effet positif sur la croissance, alors que la densité des adultes a un effet négatif sur la croissance. Les modèles basés seulement sur les facteurs dépendants de la densité prédisent les valeurs de $\bar{L}_{3m}$ et de $\bar{L}_{5f}$ de façon imprécise en 2000; l'addition des facteurs indépendants de la densité améliore les prédictions de $\bar{L}_{3m}$ et de $\bar{L}_{5f}$ des modèles. Il n'est pas possible de prédire la densité des dorés adultes à partir de leur croissance. Les patrons régionaux de croissance des dorés sont en corrélation avec les valeurs de remplacement de la productivité des lacs et, de façon moins marquée, avec la densité des adultes. Les facteurs liés à la densité ont un effet relativement faible sur les patrons de croissance, ce qui laisse croire que la croissance ne peut être utilisée comme méthode de remplacement pour suivre les changements d'abondance.

[Traduit par la Rédaction]

Introduction

Understanding the mechanisms that affect fish growth represents a challenging task for fisheries managers because of the host of abiotic, biotic, density-dependent, and density-independent factors acting to regulate fish growth. Whereas certain general characteristics of fish growth are well documented (e.g., decreased growth with decreasing temperature, decreased growth with age), other characteristics are affected by a multitude of stochastic and intra- and interspecific competitive interactions (Kitchell et al. 1977; Walters and Post 1993; Post et al. 1999). For example, the magnitude of density-dependent and density-independent factors that influence the patterns in observed growth dynamics is many times difficult to determine (Botsford 1981; Post et al. 1999). Further, age effects and sexually dimorphic growth rates may increase the uncertainty associated with growth predictions over time and space. Nevertheless, growth is easily measured, whereas estimates of fish densities and density-independent factors affecting fish populations are often expensive to collect, laborious, and potentially biased by large measurement errors (Ricker 1973; Walters and Ludwig 1981; Walters and Collie 1988).

The dynamics of walleye (*Sander vitreus*) growth and the factors influencing it have been well documented for a few individual populations (Kempinger and Carline 1977; Shuter and Koonce 1977; Staggs and Otis 1996); however, extrapolations of intralake patterns to regional-scale management have rarely succeeded. For example, walleye growth has been found to be sexually dimorphic in some lakes (Rawson 1957; Wolfert 1977; Henderson et al. 2003) but not in others (Ragan 1976; Roberge et al. 1988). In general, density-

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Table 1. Walleye (*Sander vitreus*) growth variables and density-independent predictor variables examined during model development.

Walleye growth variable	Density-independent variable
Combined sexes mean size-at-age (cm) (ages 3–12)	Maximum depth (m) (12.9, 6.8) (3.0, 35.1)
Male mean size-at-age (cm) (ages 3–10)	pH (7.2, 0.6) (5.6, 9.1)
Female mean size-at-age (cm) (ages 5–12)	Alkalinity (ppm) (37.1, 19.1) (8.0, 109.0)
	Conductance (μmho) (83.8, 35.5) (20.0, 192.0)
	MEI (11.6, 8.1) (0.9, 66.8)
	Ice-free degree-days (2628, 284) (2075, 3104)

Note: All predictors were included during best subsets regression to determine the best model of walleye growth across ceded territory lakes from 1990 to 1999. The density-independent variables include summary statistics of (mean, standard deviation) (minimum value, maximum value). MEI, morpoedaphic index.

independent factors such as climate and lake morphometry are used to explain patterns in walleye growth and population dynamics between lakes (Nate et al. 2000). Unfortunately, incorporation of these physical variables has provided little help in resolving the uncertainty associated with predicting regional-scale patterns (Walters and Collie 1988). Further, precise measurement of density-independent factors is costly where many lakes are involved, and it is not clear that obtaining such information is worth the expense.

Faced with the uncertainty and costs of identifying density-independent factors over a suite of lakes, a more fundamental and applicable concern of fisheries managers is whether walleye growth is density dependent when viewed at a regional scale. The potential effects of walleye abundance on growth are relatively easy to determine, especially when existing surveys already collect population estimates, length, weight, age, and sex data. This information can be used to determine the relative influences of density-dependent and density-independent factors on walleye growth.

Density-dependent mechanisms that shape patterns in fish growth can have important implications for developing fishery regulations or monitoring programs. For example, managers must often consider the trade-off between producing large numbers of small fish or small numbers of large fish when setting regulations to control fish densities (Walters and Juanes 1993). Density-dependent changes in growth may also be used as a surrogate method for monitoring abundance changes over many lakes. For example, a growth rate or size-at-age threshold could be used to determine whether management action, such as stocking or fishery closure, is needed to prevent overexploitation. Although the effects of stocking walleye are mixed, such growth thresholds could also forewarn managers before the collapse of a particular stock (Kempinger and Churchill 1972; Li et al. 1996*a*, 1996*b*).

In a previous study, walleye growth was density dependent within the majority of individual ceded territory (northern one third of Wisconsin, USA, containing over 800 walleye lakes) populations examined (Sass et al. 2004). However, population growth parameters such as L_∞, ω, and body condition did not vary with walleye density when viewed at a regional scale (Sass et al. 2004). Owing to the complex interactions involved with predicting adult walleye growth patterns among lakes, we examined an age-specific growth measure (mean size-at-age), separated the growth measure by sex because of the potential for sexually dimorphic growth among lakes (Henderson et al. 2003), and considered several density-independent factors in an attempt to scale up from individual-lake to regional-scale patterns.

The objectives of this study were fourfold. First, we determined which age- and sex-specific walleye growth measures were most density dependent among ceded territory populations from 1990 to 1999. Second, we developed several regression models to quantify the relative magnitude of density-dependent and density-independent processes affecting walleye growth among the selected growth measures. Third, we tested whether the regression models could accurately predict walleye growth in 2000. Lastly, we independently determined whether these walleye growth and density-independent factors could be used as a surrogate method for predicting walleye densities in ceded territory populations.

Methods

We used data collected by the Wisconsin Department of Natural Resources (WDNR) and the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) from 1990 to 1999 to determine whether walleye growth behaves density dependently and whether density-dependent processes are strong enough to use walleye growth as a surrogate method for monitoring abundance changes across ceded territory lakes. The ceded territory of Wisconsin encompasses the northern one third of the state, includes 859 lakes containing walleye populations, and has accommodated a joint tribal spearing and recreational angling fishery for walleye since 1985. The WDNR and GLIFWC have conducted total and adult population estimates (Chapman-modified Petersen estimate) and collected the length, weight, age, and sex of walleyes from a subsample of each population from a total of about 25–30 lakes·year^{-1} as part of the larger stock assessment program. A small proportion of the total lakes surveyed were sampled twice from 1990 to 1999. Adult walleye densities have ranged from just over zero to 60·ha^{-1} from 1990 to 1999. Although evidence for density-dependent growth was shown in previous studies within individual ceded territory lakes, regional patterns showed no strong signal and were highly variable (Sass et al. 2004). We hypothesized that inherent differences among ceded territory lakes (e.g., depth, productivity, climate) and the use of general growth parameters (e.g., L_∞, ω, body condition) lead to the high variability observed on a regional scale. Therefore, we considered adult density and a suite of density-independent predictor variables in the development of models to predict walleye

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growth across the ceded territory and to determine the relative contribution of each factor.

Walleye growth

We considered age-specific mean size for combined and separate sexes because walleye growth has been found to be sexually dimorphic among some lakes (Henderson et al. 2003) (Table 1). We did not consider growth measures such as L_∞, ω, body condition, or growth rate because they are unrelated to adult density among ceded territory walleye populations (Sass 2001; Sass et al. 2004). Age-specific growth measures were chosen because they result in high statistical power to detect effects of changing ecological conditions (Carpenter et al. 1995). We expected that mean sizes of the youngest age-classes would be best predicted by adult density because they were most influenced by current densities. Because sex is determined nondestructively for ceded territory walleye, we expected that the ages at first maturity (males, age 3; females, age 4 or age 5) would show the strongest density-dependent growth, if present (Sass 2001). For both combined and separate sex measures of each population, all age-classes with a sample size of three or greater were used. We examined growth measures for ages 3–12 (N: age 3 = 215 populations, age 4 = 234, age 5 = 239, age 6 = 235, age 7 = 232, age 8 = 227, age 9 = 212, age 10 = 193, age 11 = 137, age 12 = 111) for combined sexes, ages 3–10 (N: age 3 = 183 populations, age 4 = 219, age 5 = 219, age 6 = 210, age 7 = 196, age 8 = 175, age 9 = 132, age 10 = 106) for males, and ages 5–12 (N: age 5 = 179 populations, age 6 = 190, age 7 = 196, age 8 = 176, age 9 = 158, age 10 = 155, age 11 = 101, age 12 = 82) for females. Mean size-at-age was calculated for combined sexes, males, and females for all populations with a corresponding population estimate from 1990 to 1999.

Density-independent variables

We included several density-independent variables inherent to each individual lake for predictor variables during model development (Table 1). We chose a specific set of pertinent density-independent factors to minimize multicollinearity among independent variables (Graham 2003). All limnological variables chosen were temporally conservative properties of lakes (e.g., maximum depth, conductivity) and determined from a single census (Magnuson et al. 1990; Webster et al. 1996). Maximum depth was used to represent a morphometric measure of an individual lake and was gathered from the WDNR surface water indices of Wisconsin. Conductance, alkalinity, and pH were selected as chemical variables. Conductance and pH are strongly correlated with lake productivity and landscape position (Webster et al. 1996; Soranno et al. 1999). Data for conductance, alkalinity, and pH were gathered from the WDNR surface water indices of Wisconsin. Total dissolved solids for each lake was calculated as 0.67 times the specific conductance and used in the calculation of the morphoedaphic index (MEI) (Ryder 1965; Godbout and Peters 1988; Lacasse and Magnan 1992). The MEI was used to estimate the potential fish production of lakes in the ceded territory (Ryder 1965). The MEI was calculated as

$$\text{MEI} = \text{TDS}/\text{mean depth} \quad (1)$$

where TDS is total dissolved solids (0.67 × specific conductance) and mean depth (feet) was found from http://www.lake-link.com by entering the lake name and county in the lake finder feature. Water temperature is a strong predictor of walleye growth patterns (Kitchell et al. 1977). Ice-free cumulative degree-days for each year were gathered from the North Temperate Lakes Long-Term Ecological Research website (http://www.limnology.wisc.edu). We used average daily temperature at the Trout Lake Research Station, Boulder Junction, Vilas County, Wisconsin, and annual ice-on and ice-out dates from Big Muskellunge Lake, Vilas County, Wisconsin. Cumulative degree-days were the sum of the annual ice-free degree-days, where 1 degree-day equaled 1 °C above 0 °C for 24 h (Ketola et al. 2000). In our analysis, ice-free degree-days in the year prior to the population sampling were used to predict growth.

Model selection

We regressed each growth measure separately on adult density to determine the magnitude of density dependence, if present at all, across all ceded territory populations sampled from 1990 to 1999. We tested for density dependence to establish the logical connection between growth and density prior to predicting density from growth. Because density is likely to be independent of growth, we established density-dependent growth prior to reversing the relationship for predictive purposes. The growth measures best predicted by adult density were chosen for further analysis to quantify the magnitude of density-dependent and density-independent processes affecting walleye growth across the region. We used best subsets regression with the full compliment of density-independent variables to determine the best predictors of walleye growth. A Bonferroni correction was applied to reduce Type I error associated with the large number of comparisons (N = 26) before consideration for final model selection. Final model consideration was given at the α = 0.0019 (0.05/26 comparisons) level following the Bonferroni correction (Rice 1989). Final model selection was conducted using Mallow's Cp statistic and r^2 values. Mallow's Cp statistic is calculated by

$$\text{Cp} = \text{Rss}_p/s^2 - (n - 2p) \quad (2)$$

where Rss_p is the residual sum of squares from a model containing p parameters, p is the number of parameters in the model including β_0 (the y intercept of the regression line), and s^2 is the mean square (sample variance) from the largest equation postulated containing all of the predictors (Draper and Smith 1998). The best models of walleye growth were selected when the absolute difference between Cp and p was minimized and the r^2 values were maximized (Furnival 1971; Neter et al. 1996; Draper and Smith 1998). After the best model was selected for each growth measure, all interactions of the predictor variables were examined and dropped from the model if the t value exceeded α = 0.05. Lastly, partial r^2 was determined separately for each predictor to quantify the magnitude of density-dependent and density-independent processes and each predictor's contribution to the final model.

The adult walleye density estimates, growth metrics, and density-independent predictor variables used in this study have the potential to be plagued by variable degrees of mea-

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surement error inherent to fisheries data (Ricker 1973; Walters and Ludwig 1981; Hilborn and Walters 1992). In such cases, an errors-in-variables model is appropriate under certain conditions of the data and statistical test used. Despite potential measurement error, an errors-in-variables model was either not necessary or inappropriate for this study. According to Hilborn and Walters (1992), predictor variables that vary no more than two to four times from lowest to highest generally will result in severe bias. However, if the smallest value observed is at least 1/10th of the largest value, biases will probably be of little concern (Hilborn and Walters 1992). The lowest adult density estimate used in this study was 1/60th of the highest observed density and most adult densities ranged from 0 to 10 walleye·ha^{-1} (Sass et al. 2004). The smallest values observed for our density-independent variables were also at least 1/10th of the largest values observed (Table 1). Hilborn and Walters (1992) also stressed the importance of reliability in population estimates. Any density estimate with a coefficient of variation exceeding 10% was excluded from our analyses.

Use of functional regression proposed by Ricker (1973) and other statistical methods for dealing with measurement error (Ludwig and Walters 1981; Walters and Ludwig 1981) were also inappropriate for our purposes. Functional regression requires a one-predictor model, where the predictor variable is not a mean value (Ricker 1973). Our analysis was not conducive for functional regression because the growth predictor variable examined was mean size-at-age. In addition, multiple predictors were required to quantify the contribution of density-dependent and density–independent measures influencing walleye growth. More complicated approaches for dealing with measurement error (Ludwig and Walters 1981; Walters and Ludwig 1981) have been proposed but are complicated and do not work extremely well (Hilborn and Walters 1992; Quinn and Deriso 1999).

Walleye growth model predictions

We used independent data collected by the WDNR in 2000 to examine the predictive capabilities of the adult density and full models separately. For each population sampled in 2000, growth measures were calculated and observed walleye growth was compared with the predicted walleye growth from each of the models. The ability of the models to predict walleye growth was evaluated by calculating the mean absolute difference between predicted and observed walleye growth. The predictive capacity of these models assumes that adult walleye density remains constant over time. Ceded territory walleye densities among lakes, regardless of the coefficient of variation in the population estimate, did not change during 1990 to 1999 (Sass et al. 2004). Similarly, we observed no changes in adult densities over consecutive 3- to 5-year study periods of walleye populations when a 10% coefficient of variation was considered the maximum acceptable standard of population estimate variability (G.G. Sass, unpublished data).

Adult walleye density model predictions

We used the walleye growth measures best predicted by adult density and the density-independent characteristics to determine if (*i*) adult walleye densities could be predicted accurately in 2000 using the full compliment of data available from 1990 to 1999 and (*ii*) randomly selecting half of the populations available from 1990 to 2000 to create a model and then using the other half of the populations to validate the model resulted in better predictions of adult density. We used best subsets regression, independent of the previously established growth models, to determine the final models from the suite of growth measures and density-independent characteristics because of the potential for density to be unrelated to factors predicting growth. We did not use inverse prediction (i.e., an algebraic rearrangement of the growth models) to determine adult densities from the growth models because the procedure assumes dependence of all variables in the model (Zar 1984). Instead, we independently tested the predictors of growth and adult density without assuming dependence among the variables (Zar 1984). For our randomly selected population model, we also attempted to increase model predictions by randomly selecting one lake-year when multiple years of data existed for the same walleye population to avoid bias from potential lack of independence. Model predictions were tested against the observed adult density in a given lake-year. We considered the model prediction to be reasonable if the predicted adult density fell within the 95% confidence intervals of the observed density.

Results

The mean size of age-3 males ($\bar{L}_{3m}$) (centimetres) and age-5 females ($\bar{L}_{5f}$) (centimetres) was best predicted by adult density from 1990 to 1999. The $\log_e$ of adult density (A) (walleye per hectare) explained 12% of the variability in $\bar{L}_{3m}$ ($F = 25.15$, df = 1,180, $p < 0.001$, $N = 182$) (Fig. 1*a*). The relationship between $\bar{L}_{3m}$ and adult density was best represented by the model

$$(3) \qquad \bar{L}_{3m} = 32.784(\pm 0.64) - 1.477(\pm 0.3)\ln A$$

Standard errors of the regression coefficients in eq. 3 and all proceeding models are given in parentheses. Adult density was $\log_e$-transformed to establish normality in the independent variable (Draper and Smith 1998). The $\log_e$ of adult density explained 13% of the variability in $\bar{L}_{5f}$ from 1990 to 1999 ($F = 26.84$, df = 1,176, $p < 0.001$, $N = 178$) (Fig. 1*b*). The model

$$(4) \qquad \bar{L}_{5f} = 45.796(\pm 0.97) - 2.317(\pm 0.45)\ln A$$

best described the relationship between $\bar{L}_{5f}$ and adult density.

The amount of variability explained in $\bar{L}_{3m}$ and $\bar{L}_{5f}$ increased almost threefold when density-independent predictors were included (Tables 2 and 3). The $\bar{L}_{3m}$ was best described by the model

$$(5) \qquad \bar{L}_{3m} = 18.708(\pm 3.3) + 1.5201(\pm 0.45)\text{pH} - 0.9629(\pm 0.38)\ln A + 0.2551(\pm 0.1)\text{MEI} + 0.08707(\pm 0.04)\text{maximum depth}$$

The combination of pH, $\log_e$ adult density, MEI, and maximum depth explained 33% of the variability in $\bar{L}_{3m}$ ($F = 11.49$, df = 4,92, $p < 0.001$, $N = 96$). The best predictors of $\bar{L}_{5f}$ were pH, $\log_e$ adult density, and conductance, which ex-

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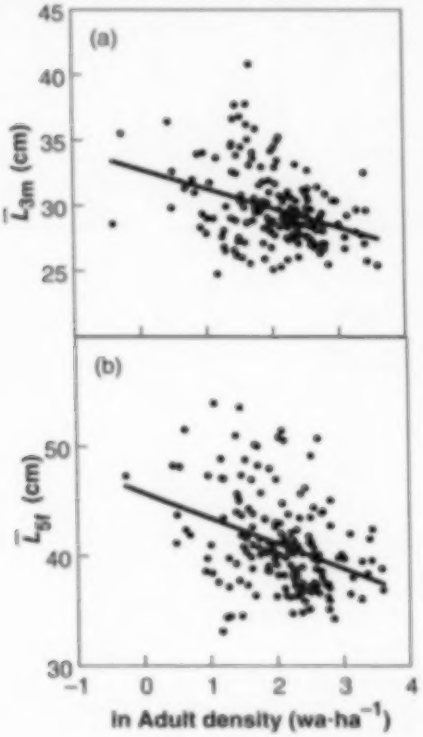
Fig. 1. Relationship between the mean size of (*a*) age-3 male walleye (wa; *Sander vitreus*) ($\bar{L}_{3m}$) ($r^2 = 0.12$, $p < 0.001$) and (*b*) age-5 female walleye ($\bar{L}_{5f}$) ($r^2 = 0.13$, $p < 0.001$) and $\log_e$ adult walleye density in ceded territory lakes during 1990 to 1999.



plained 31% of the total variability ($F = 13.28$, df = 3,89, $p < 0.001$, $N = 93$). The full model was given by

(6) $\bar{L}_{5f} = 31.401(\pm5.5) + 1.5236(\pm0.8)\text{pH} - 2.1598(\pm0.6)\ln A + 0.03834(\pm0.01)\text{conductance}$

The single best predictor of $\bar{L}_{3m}$ and $\bar{L}_{5f}$ was pH, which accounted for 20% and 18% of the variability explained in each model, respectively (Tables 2 and 3). The $\log_e$ of adult density explained an additional 6% in model 5 and 8% in model 6. Conductivity provided an additional 5% to model 6, while MEI and maximum depth contributed 3% and 4%, respectively, to model 5. The additional density-independent predictors contributed little to each model's overall performance (Tables 2 and 3). Separation of the derived MEI variable components (total dissolved solids, mean depth) into independent variables did not improve model fits.

The $\bar{L}_{3m}$ and $\bar{L}_{5f}$ in 2000 were not predicted from adult density. Models 3 and 4 always overestimated mean sizes at lower abundances and underestimated mean size at higher abundances. Overall, models 3 and 4 had a mean absolute difference of 1.85 and 3.51 cm, respectively, between predicted and observed mean size-at-age in 2000.

Models 5 and 6 predicted the $\bar{L}_{3m}$ and $\bar{L}_{5f}$ reasonably well in 2000 (Figs. 2 and 3). The mean absolute difference between the predicted and observed $\bar{L}_{3m}$ was 1.3 cm. Model 6 performed slightly worse and resulted in a mean absolute difference of 2.71 cm between predicted and observed mean sizes.

Adult densities from 1990 to 1999, using all data, were best predicted by the model

(7) $\ln A = 4.37(\pm0.62) - 0.0766(\pm0.02)\bar{L}_{3m}$

The $\bar{L}_{3m}$ explained 13% of the variability in $\log_e$ adult density from 1990 to 1999 ($F = 13.58$, df = 1,93, $p < 0.001$, $N = 94$). Model 7 did not predict adult densities accurately in 2000; only 8 of 21 (38%) predictions fell within the 95% confidence intervals of observed adult density (Fig. 4). When half of the populations were randomly selected and multiple lake-years were removed, model predictions improved slightly. Adult densities of walleye not used to create the model were best predicted by the model

(8) $\ln A = 5.05(\pm0.8) - 0.0997(\pm0.03)\bar{L}_{3m}$

For model 8, 21% of the variability in $\log_e$ adult density was explained by $\bar{L}_{3m}$ ($F = 13.99$, df = 1,54, $p < 0.001$, $N = 55$). Model 8 predicted 21 of 53 (40%) adult densities within the 95% confidence interval of the observed density (Fig. 5). All female models considered were poor predictors of adult densities and not considered further. Previously identified density-independent predictor variables of growth did not significantly contribute ($p > 0.05$) to model 7 or 8 when predicting density.

Discussion

The incorporation of age-specific growth measures and the separation of sexes were important in showing stronger evidence for density-dependent growth across ceded territory walleye populations. General growth parameters, which disregarded sex, inherently introduced variability into the growth relationships because walleye growth was sexually dimorphic in the ceded territory (Fig. 6). The ability to collect and identify individual sexes nondestructively is highly seasonal, as most fishes do not display sexual dimorphism morphologically. In general, sexual dimorphism can only be observed by spawning coloration (e.g., darters, Percidae), morphology (e.g., Salmonidae, male kype; Catostomidae, male nuptial tubercles), or parental guarding behavior (e.g., Centrarchidae, Cichlidae) or through artificial release of the gametes during spawning (e.g., walleye, salmon), making sexual determination difficult or seasonal at best. Sexually dimorphic growth of walleye in individual lakes has been shown in previous studies (e.g., Rawson 1957; Wolfert 1977), but rarely as a large-scale and long-term regional phenomenon (Henderson et al. 2003) (Fig. 6). If possible, sexually dimorphic growth should be acknowledged when examining fish population dynamics.

The $\bar{L}_{3m}$ and $\bar{L}_{5f}$ decreased exponentially with increases in adult walleye densities. This density-dependent relationship for walleye contrasted Post et al. (1999) and Diana et al. (1991) who found linear decreases in adult rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*) growth, respectively, with increasing density. The linear density-dependent growth observed for Nile tilapia may have differed because growth rate (grams per day) was used or because densities were not extreme enough to test for exponential decay in Nile tilapia growth (Diana et al.

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Table 2. Results from best subsets regression for predictors of $\bar{L}_{3m}$ walleye (*Sander vitreus*) from 1990 to 1999 with the corresponding number of predictors (P), the regression coefficients with standard errors in parentheses, overall r^2, and partial r^2 contributed by the additional predictor.

P	pH	ln walleye·ha^{-1}	MEI	Maximum depth (m)	Alkalinity (ppm)	Degree-days	Conductance (μmho)	r^2	Partial r^2
1	**2.192**							0.204	0.204
2	**1.983**	**–1.0838**						0.266	0.062
3	**1.799**	**–1.0217**	**0.1975**					0.294	0.028
4	**1.52 (±0.45)**	**–0.9629 (±0.38)**	**0.2551 (±0.1)**	**0.08707 (±0.04)**				0.333	0.039
5	**1.284**	**–0.9838**	**0.2234**	**0.08113**	–0.01573			0.336	0.003
6	**1.272**	**–0.9906**	**0.2223**	**0.08204**	–0.01608	0.0003		0.337	0.001
7	**1.276**	**–0.9854**	**0.2263**	**0.08151**	–0.01885	0.0003	0.0019	0.337	0

Note: Coefficients in bold denote significant predictors at the $\alpha = 0.05$ level. MEI, morpoedaphic index.

Table 3. Results from best subsets regression for predictors of $\bar{L}_{5f}$ walleye (*Sander vitreus*) from 1990 to 1999 with the corresponding number of predictors (P), the regression coefficients with standard errors in parentheses, overall r^2, and partial r^2 contributed by the additional predictor.

P	pH	ln walleye·ha^{-1}	Conductance (μmho)	Degree-days	Alkalinity (ppm)	MEI	Maximum depth (m)	r^2	Partial r^2
1	**3.051**							0.178	0.178
2	**2.652**	**–2.0001**						0.258	0.08
3	**1.524 (±0.8)**	**–2.1598 (±0.6)**	**0.03834 (±0.01)**					0.309	0.051
4	**1.577**	**–2.0762**	**0.03772**	0.0022				0.332	0.023
5	**1.868**	**–2.1314**	**0.07552**	0.002	0.07568			0.337	0.005
6	**1.868**	**–2.0983**	**0.06648**	0.0021	0.06364	0.074		0.338	0.001
7	**1.702**	**–1.9632**	**0.06162**	0.002	0.05834	0.1175	0.05123	0.344	0.006

Note: Coefficients in bold denote significant predictors at the $\alpha = 0.05$ level. MEI, morpoedaphic index.

Fig. 2. Predicted mean size of age-3 male walleye (*Sander vitreus*) ($\bar{L}_{3m}$) from the model $\bar{L}_{3m}$ (cm) = 18.708 + 1.5201 pH – 0.9629 ln adult density (walleye·ha^{-1}) + 0.2551 MEI + 0.08707 maximum depth (m) versus observed $\bar{L}_{3m}$ in 2000. The line represents the 1:1 line corresponding to equivalent predicted and observed $\bar{L}_{3m}$. MEI, morpoedaphic index.

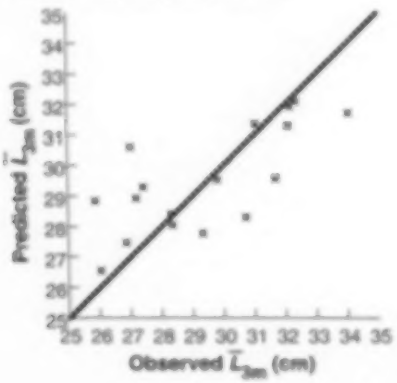
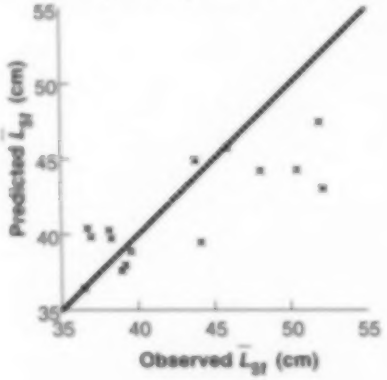


Fig. 3. Predicted mean size of age-5 female walleye (*Sander vitreus*) ($\bar{L}_{5f}$) from the model $\bar{L}_{5f}$ (cm) = 31.401 + 1.5236 pH – 2.1598 ln adult density (walleye·ha^{-1}) + 0.03834 conductance (μmho) versus observed $\bar{L}_{5f}$ in 2000. The line represents the 1:1 line corresponding to equivalent predicted and observed $\bar{L}_{5f}$.



1991). An exponential decrease in rainbow trout growth may not have been observed because effective density (square centimetres per hectare × 10^{-5}) was used instead of actual density (Post et al. 1999).

Measurement error associated with our growth measures may have reduced our ability to detect stronger patterns in density-dependent growth. While our smallest adult density estimates were at least 1/10th of our largest estimates, measurement error in the population estimates would act to make growth look to be less affected by adult density than it really is (Hilborn and Walters 1992). If this was the case, our correlations between walleye growth and density may have been even stronger than those observed. However, our range of values in adult walleye densities among lakes and our standard of only using estimates with coefficients of variation ≤10% should have been sufficient to avoid bias (Hilborn and Walters 1992).

The measure of pH was the best predictor of $\bar{L}_{3m}$ and $\bar{L}_{5f}$. The primary productivity of lakes, fish productivity and

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Fig. 4. Predicted adult walleye (wa; *Sander vitreus*) density from the model ln adult density (wa·ha^{-1}) = 4.37 – 0.0766 $\bar{L}_{3m}$ (cm) versus observed adult walleye density from lakes in 2000. The line represents the 1:1 line corresponding to equivalent predicted and observed adult walleye density. Predicted adult density is denoted by triangles. Ninety-five percent confidence intervals for observed density are represented by circles.

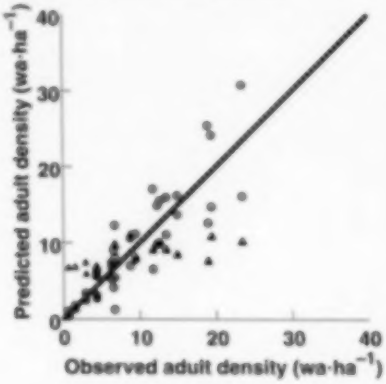
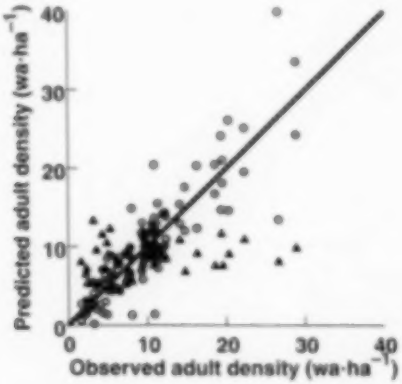


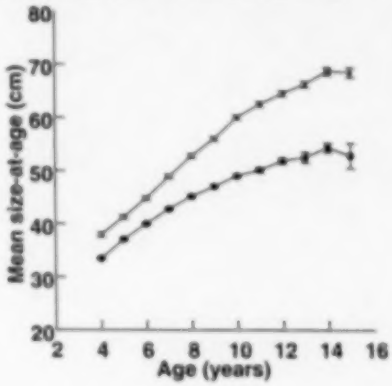
Fig. 5. Predicted adult walleye (wa; *Sander vitreus*) density from the model ln adult density (wa·ha^{-1}) = 5.05 – 0.0997 $\bar{L}_{3m}$ (cm) versus observed adult walleye density from lakes not selected for model development. The line represents the 1:1 line corresponding to equivalent predicted and observed adult walleye density. Predicted adult density is denoted by triangles. Ninety-five percent confidence intervals for observed density are represented by circles.



growth, and lake landscape position are often correlated with pH (Kelso and Johnson 1991; Hubert and Chamberlain 1996; Webster et al. 1996). For example, pH is positively correlated with rainbow trout mean size-at-age (Hubert and Chamberlain 1996). If pH can be used as a surrogate measure of lake productivity, our models suggest that increased lake productivity has a positive effect and is a key factor influencing walleye growth.

Conductance increased the variability explained in and had a positive influence on $\bar{L}_{5f}$. Conductivity is a conservative chemical property of lakes and has also been positively correlated with lake productivity in northern Wisconsin (Magnuson et al. 1990; Soranno et al. 1999). Conductivity is

Fig. 6. Male and female walleye (*Sander vitreus*) mean size-at-age from all lakes sampled in the ceded territory, 1990 to 1999. Female walleye are denoted by crosses and males by circles. Error bars represent ± standard error of the mean.



positively correlated with the mean size of lake trout (*Salvelinus namaycush*) (Trippel and Beamish 1989). Although conductivity was not influential in determining $\bar{L}_{3m}$, the MEI was. The MEI provides a measure of fish production from a given lake and is derived from morphometric characteristics of individual lakes (mean depth) and a surrogate measure of productivity, total dissolved solids (Ryder 1965; Hubert and Chamberlain 1996). The MEI had a positive influence on male growth, further suggesting that lake productivity has an important influence on walleye growth. Increases in productivity should positively influence walleye growth by increasing food abundance. Indeed, similar effects of increasing productivity on fish biomass and growth have been shown experimentally for kokanee salmon (*Oncorhynchus nerka*) and Arctic grayling (*Thymallus arcticus*) through the artificial fertilization of oligotrophic systems (Deegan and Peterson 1992; Budy et al. 1998; Gross et al. 1998).

The incorporation of density-independent predictors into the full models improved predictions of the $\bar{L}_{3m}$ and $\bar{L}_{5f}$ dramatically over the sole predictor of adult density. However, predicted mean size-at-age was still variable in many cases. This is likely a function of the overall lack of variability explained by each full model, which was roughly only one third of the total variability in $\bar{L}_{3m}$ and $\bar{L}_{5f}$. Further, male mean size-at-age was likely better predicted because the variance in mean size of females is about three times greater than in that of males across all age-classes from 1990 to 1999. Clearly, productivity measures and adult density comprise a portion of the factors explaining walleye growth across the ceded territory, but they are not the only factors.

We expected that the inclusion of ice-free degree-days would contribute to the overall models because water temperature has been found to have a large influence on walleye growth (Kitchell et al. 1977). The calculation of degree-days for each year from a fixed point in northeastern Wisconsin may not have been representative of the entire ceded territory. For example, lakes in the northwestern part of the ceded territory generally thaw earlier than lakes in the northeastern part presumably leading to more ice-free degree-days. The lack of individual lake ice-on and ice-off data

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from the entire ceded territory may have hindered this variable's influence in the overall models.

The forage base present within each lake could also have an influence on walleye growth. For example, yellow perch (*Perca flavescens*) and gizzard shad (*Dorosoma cepedianum*) abundance has been correlated with walleye growth and recruitment in previous studies (Forney 1974; Madenjian et al. 1996; Hansen et al. 1998). Non-native rainbow smelt (*Osmerus mordax*), which are present in some Wisconsin lakes, also have a strong, positive influence on walleye growth (Jones et al. 1994; Johnson and Goettl 1999). Yellow perch catch and harvest rate data exist for some ceded territory lakes from creel surveys in 1990 to 1999. We attempted to use these variables to represent a forage base predictor of walleye growth; however, they did not explain any additional variability in the $\bar{L}_{1n}$ and $\bar{L}_{5t}$ and were excluded. Nevertheless, the forage base of individual lakes may still have been an important factor influencing walleye growth if larger sample sizes and more accurate measures of forage base population abundance were available.

Adult density in 2000 and in randomly selected lakes from 1990 to 2000 was not predicted accurately by walleye growth in models 7 and 8. Further, no density-independent characteristics improved the amount of variability explained by walleye growth in each model, which supports our assumption of independence between all variables and our decision to not use inverse prediction as a biologically relevant statistical test (Zar 1984). This may suggest that adult walleye density is not influenced by density-independent characteristics of the lakes themselves; however, Nate et al. (2000, 2001) has shown that increased lake size, percentage of sand and muck bottom substrate, and conductivity have a positive influence on walleye abundance. There are several sources of error in the independent variables that may have accounted for our lack of ability to predict adult walleye density on a regional scale including error associated with the population estimates, timing of the population estimates, and stocking (Ricker 1973; Walters and Ludwig 1981). The WDNR and GLIFWC conduct about 25–30 total population estimates on ceded territory lakes each year. Variability in electroshocker boat speed and drivers during mark and recapture runs as well as its influence on the ability to dipnet walleye when abundant could be a large source of potential error in the population estimates (underestimate). Further, many lakes in northern Wisconsin harbor midlake spawning reefs that cannot be sampled by electroshocking. Population estimates in the ceded territory are conducted from ice-out (early April) until late May. Because electroshocking effort focuses on the entire shoreline of lakes, the timing of the sample could drastically alter the population estimate. For example, fewer walleye may be present in shallow spawning grounds in late May compared with late April. Lastly, lags in growth responses following stocking may have influenced walleye growth patterns substantially. For example, many lakes jump from low densities of walleye to high densities in a short amount of time as a result of stocking. This may result in walleye growth patterns that are not reflective of density. Based on the results of this study, it is clear that adult density influenced walleye growth; however, the magnitude of this effect is either weak or a strong pattern could not be detected because of error associated with population and growth estimates. Nevertheless, the range of density estimates used in these analyses should have been sufficient to avoid potential bias (Hilborn and Walters 1992). Despite the shortcomings of the sampling protocol, the current stock assessment program must continue in this fashion to calculate harvest quotas for the tribal spearing and recreational angler walleye fisheries (Hansen et al. 1991). Because the protocol has not changed since 1990 and minimum tolerance limits of variability were established for the estimates used in this analysis, we feel that our results provide a good representation of the factors influencing walleye growth in ceded territory lakes.

The ultimate goal of this study was to determine whether walleye growth could be used as a surrogate method for monitoring abundance changes across ceded territory walleye populations. The use of walleye growth to measure abundance changes relies on the assumption that density-dependent processes dominate walleye growth patterns among ceded territory lakes. This study suggests that adult density does influence regional walleye growth patterns; however, the magnitude of density dependence is insufficient to track changes in adult walleye growth accurately as evidenced by the poor capabilities of models 3 and 4 to predict growth in 2000. The addition of density-independent and adult density variables into models 5 and 6 improved the predictions of walleye growth in 2000, but not sufficiently. Adult density was not predicted well in 2000 or in randomly selected lakes using walleye growth parameters. Clearly, a different suite of variables such as exploitation, availability of spawning habitat, fish species composition (i.e., food web effects, predator–prey interactions), and forage bases in individual walleye populations may be driving patterns in walleye densities independently of factors influencing regional walleye growth. At this time, walleye growth cannot be used to monitor walleye abundance changes. However, we stress that our models and modeling approach should not be taken as final, and continual updating and revalidation should proceed when additional data become available. Lake productivity appears to be the dominant component of regional-scale walleye growth dynamics. Density dependence is also important but is secondary to density-independent factors. A more extensive lake-specific examination of density-independent factors (e.g., forage base and degree days) may be necessary to explain additional variability in walleye growth before growth dynamics could be used as a management option for monitoring abundance changes.

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Catch-up growth and swimming performance in threespine sticklebacks (*Gasterosteus aculeatus*): seasonal changes in the cost of compensation

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Abstract: Compensatory growth is a phase of unusually rapid growth following a period of growth depression. This response allows animals to achieve the same size-for-age as continuously fed contemporaries. We tested the hypothesis that a period of poor growth followed by compensation reduces the swimming abilities of threespine sticklebacks (*Gasterosteus aculeatus*), even after the period of rapid growth had ceased; we also investigated how the time of year in which the rapid growth occurred affected the possible costs in swimming performance. The threespine sticklebacks showed a compensatory growth response after a period of food shortage, both in winter and in spring. This change in growth trajectory subsequently caused a reduction in ability to withstand high flows, but only when the period of rapid growth occurred just prior to the breeding season. We suggest that threespine sticklebacks may be willing to incur the swimming costs of catch-up growth just prior to the breeding season to maximize their expected reproductive success.

Résumé : La croissance compensatrice consiste en une phase de croissance particulièrement rapide qui suit une période de croissance ralentie. Cette réaction permet aux animaux d'atteindre la même taille en fonction de l'âge que leurs contemporains qui se sont nourris sans interruption. Nous avons éprouvé l'hypothèse qui veut q'une période de croissance ralentie suivie d'une période de compensation réduise les capacités de nage des épinoches à trois épines (*Gasterosteus aculeatus*), même une fois la période de croissance rapide terminée; nous avons aussi déterminé si la période de l'année durant laquelle se produit la croissance accélérée affecte les coûts possibles en ce qui a trait à la performance de nage. Les épinoches à trois épines ont une réaction de croissance compensatrice après une période de pénurie alimentaire, tant en hiver qu'au printemps. Cette modification de la trajectoire de croissance réduit subséquemment la capacité à supporter les débits élevés, mais seulement lorsque la période de croissance rapide précède immédiatement la saison de reproduction. Nous croyons que les épinoches à trois épines sont prêtes à subir les coûts associés à la nage dus à la croissance compensatrice juste avant la période de reproduction afin de maximiser le succès attendu de leur reproduction.

[Traduit par la Rédaction]

Introduction

It is generally assumed that growth patterns in most animals are preprogrammed and relatively inflexible (Roff 1992; Stearns 1992). In organisms with high juvenile mortality rates, rapid growth can reduce the time spent in vulnerable life stages, thereby increasing survivorship (Arendt 1997; Sogard 1997). Moreover, animals that are large for their age often have higher social status (Martín and Forsman 1999; East and Hofer 2001), greater competitive ability (Johnsson 1993; Wada 1993), and an early age of maturation (Rowe and Thorpe 1990). Therefore, we might expect that organisms should grow as fast as possible. However, it has been demonstrated that some organisms are capable of reacting after periods of food shortage by accelerating their rate of growth above that of steadily growing individuals (Wilson and Osbourn 1960; Jobling et al. 1993). This ability to catch up in size indicates that normal growth rates are less than the maximum possible. This suggests that there are costs associated with rapid growth rates, such that there is a trade-off between the benefits of a large body size and the costs of achieving it (Arendt 1997; Metcalfe and Monaghan 2001).

High rates of growth associated with compensatory growth may be associated with greater risks of developmental abnormalities (Arendt 1997; Robinson and Wardrop 2002), increased risk-taking (Werner and Anholt 1993; Munch and Conover 2003), and aggression (Nicieza and Metcalfe 1999) and also with a reduced life span (Metcalfe and Monaghan 2003). In fish, there is also evidence that rapid growth can

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influence muscle cellularity and development (Galloway et al. 1999; Johnston et al. 2002), which may influence swimming ability.

Prolonged swimming (also called swimming stamina) is generally considered to be a measure of aerobic capacity (Beamish 1978; Kolok 1999) and hence is a good measure of the physical fitness of the fish. In the Atlantic silverside (*Menidia menidia*), faster-growing individuals had a poorer prolonged and burst swimming performance (Billerbeck et al. 2001), suggesting that there was a trade-off between growth rate and swimming performance. It is possible that individual fish that are small for the time of year may compromise on allocation of resources to muscular development to increase their skeletal growth. Previous studies have compared swimming in fish that have been growing either fast or slow at the time of the test (Kolok and Oris 1995; Billerbeck et al. 2001). As a result, the exact mechanism is unknown, and so it is not clear if the effects persist beyond the end of the period of rapid growth (as would be the case, for instance, if muscle structure was impaired). Moreover, the fastest growth rates measured in these studies were within the range of normal growth rates rather than the higher rates occurring during catch-up growth.

Fish provide one of the best model systems for studying the causes and consequences of compensatory growth (Ali et al. 2003). Body size is strongly correlated with overwinter juvenile survival in many temperate species of freshwater fish (Toneys and Coble 1979) and is also a key determinant in reproductive success (Wootton 1999). There can therefore be strong selection pressures on fish to achieve a relatively large size by fixed key times (e.g., the onset of winter or the spawning season; Nicieza and Metcalfe 1997). The response to a period of reduced food availability and the costs of any compensation may therefore vary according to the time of year.

The aim of the present study was to investigate whether the compensatory response resulted in impaired swimming performance even after the period of rapid growth had ceased and whether any impairment depended on the time of year in which the rapid growth occurred. Threespine sticklebacks (*Gasterosteus aculeatus*) were chosen for this study, since their life cycle suggests that they should be sensitive to variation in body size trajectory. Moreover, there is also strong size-dependent breeding success (Kraak and Bakker 1998; Kraak et al. 1999). Many populations are annual in that all breeding fish were born in the previous summer. There will therefore be a strong selection pressure on juvenile fish to compensate for any period of poor growth so that they achieve a suitable size by the time of the breeding season. It is already documented that threespine sticklebacks have the capability to exhibit compensatory hyperphagia and growth after food shortage (Ali et al. 1998), and they show adaptive differences in growth rates between populations (Snyder 1991), suggesting that they are capable of adaptive flexibility in growth patterns.

Materials and methods

Fish and rearing conditions

The first group of threespine sticklebacks was captured with dip nets in the River Endrick, Scotland, UK (56°04′N, 4°23′W), between 10 and 13 November 2003. A second group of fish was captured at the same location on 3 February 2004. This is an annual population, the overwhelming majority of fish dying after a single spawning season (N.B. Metcalfe, personal observation). After capture, all fish were transferred to 80-L acclimatization aquaria (90 cm × 39.5 cm × 22.5 cm) at a density of 2 fish·L^{-1} and fed ad libitum with frozen chironomid larvae. The temperature was maintained at 13.7 ± 0.3 °C throughout the experiments, while the photoperiod was adjusted weekly to match the natural photoperiod in the original river. We added 125 mL of seawater per tank to prevent the risk of whitespot (*Ichthyophthirius multifiliis*) infection.

Growth manipulation

To study the effects of compensatory growth in swimming performance, we manipulated the growth trajectories in two different seasons (winter and spring). In both experiments, fish were held in groups, since juvenile sticklebacks naturally live in shoals.

Experiment 1. Winter growth

On 3 December 2003, the fish were anaesthetized, measured for standard length (±0.01 mm) and wet mass (±0.001 g), and held in groups of five fish in 15-L tanks. Fish were randomly assigned to each tank to avoid differences in size at the beginning of the experiment. Each tank was provided with an individual filter, a gravel substrate, and artificial plants to provide refuges and reduce the stress on the fish.

Ten tanks were randomly allocated to the restricted food (RF) treatment and another 10 with the same number of fish were assigned as the control (C) group. In the RF tanks, the fish were given an amount of frozen chironomids that would keep growth rates close to zero (2% of their initial body weight per day). The C group was fed ad libitum (~10% of body weight per day, which has been found to produce maximum growth rates when sticklebacks were fed on Tubifex or enchytraeid worms (Allen and Wootton 1982)).

The fish were remeasured every 2–3 weeks; all fish were starved for 24 h prior to measuring to prevent variation in the weight of stomach contents. We stopped the growth treatment after 5 weeks, at which point the differences in size between the two groups of fish were significant (see Results). All fish were then fed ad libitum to induce catch-up growth in the RF group.

Experiment 2. Spring growth

On 15 February 2004, the second group of sticklebacks were anaesthetized, measured, and randomly allocated into groups of five fish in 15-L tanks as described for experiment 1. In this experiment, we randomly assigned tanks to three treatments: C, RF, and deprived food (DF) (n = 7 tanks of 5 fish per treatment). The rations of the C and RF treatments were the same as in experiment 1. In the DF treatment, no food was added during the manipulation period. The period of manipulation finished after 3 weeks, when there were significant size differences between the three treatment groups (see Results). Thereafter, all fish were fed ad libitum to induce catch-up growth in the RF and DF groups.

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Swimming stamina

Fish can only maintain anaerobic burst swimming for very short periods (<20 s; Kolok 1999). Measurements of swimming performance against a steady current for longer than this time are defined as measures of prolonged swimming or swimming stamina (Kolok 1999). In our experiments, the minimum period that a fish swam against the test current was 40 s (see below), and so henceforth, we describe the tests as measuring swimming stamina.

In experiment 1, swimming stamina was tested between 24 and 27 March 2004, just after full growth compensation (see below), and again 2 months later (between 19 and 21 May). Stamina was measured in experiment 2 at the end of the growth manipulation (7–9 April) as well as when the fish had achieved full growth compensation (27–29 May).

The swimming chamber consisted of a cylindrical transparent tube (250 mm long and 30 mm internal diameter) with both ends closed by 1-mm mesh. The chamber was submerged in water within a 50 cm × 30 cm × 30 cm glass aquarium and was connected to a pump to produce longitudinal water current. The pump had a valve with two positions to allow a switch in flow from slow to fast. One fish at a time was placed into the swimming chamber and exposed for 5 min to a water flow of 4.08 cm·s^{-1} (equivalent to 1 body length·s^{-1} for the mean size of the tested fish). This allowed the test fish to orient towards the water inflow and to acclimatise to the swimming chamber. After this initial period, the velocity of the water was increased in one step to 20.4 cm·s^{-1} (an average of 5 body lengths·s^{-1}, selected after preliminary trials) and time until fatigue was registered. Fatigue was defined as when the fish suddenly stopped swimming and was carried back to the downstream mesh, where it remained even when the mesh was gently prodded. At this point, the water pump was immediately turned off and the fish removed from the tube and placed in still water. All fish quickly recovered and were swimming normally again within 2–3 min. The experiments were conducted inside a controlled-temperature room that maintained the temperature at 13.7 ± 0.3 °C. The effects of turbulence on swimming performance with this kind of experimental setup are negligible and therefore do not influence the results obtained (Nikora et al. 2003). This technique has been used successfully to measure swimming stamina in juvenile brown trout (*Salmo trutta*) (see Ojanguren and Braña 2000, 2003). In each experiment, we tested three fish per tested tank (in Experiment 1, we did 42 swimming tests (7 tanks × 3 fish × 2 treatments) per season and 63 tests in experiment 2 (7 tanks × 3 fish × 3 treatments) per season). After the tests, each fish was measured, weighed, and then returned to its holding tank.

Statistical analysis

To prevent pseudoreplication, we used the average of the mass or length of all of the fish of each tank at each sampling time as the variable to analyse growth. Some mortality occurred during experiment 1 (see below), so we used only those tanks (seven from each treatment) that had sufficient surviving fish (three or more) for the analysis; in such cases, tank means were based on surviving fish. The total number of fish used in the analysis was therefore 58 fish (30 C fish and 28 RF fish). In experiment 1, we measured the fish twice during the growth manipulation period and four times during the compensation phase. In experiment 2, the fish were measured once at the end of the manipulation period and five times during the recovery phase. The total number of fish used in the analyses was 92 (105 initial fish minus 13 fish that died during the experiment: 32 C fish, 29 RF fish, and 31 DF fish). We used multivariate analysis of variance (MANOVA) to test for overall differences in body length and mass at each sampling time. In addition, the growth trajectories of control and growth-manipulated fish were compared by repeated-measures ANOVA (RM-ANOVA). The subject was the tank, ration level was a between-subjects factor, and the repeated measures of length or mass at different times were a within-subjects factor. Differences in growth rates of the RF treatments during the compensation period between winter and spring growth experiments were evaluated by analysis of covariance (ANCOVA) of the length increments during this period using length at the start of that interval as a covariate. To analyse differences in swimming performance between the food deprivation treatments, we used a nested ANCOVA using the treatment as a fixed effect, tanks as a random nested effect, and length as a covariate. We used Cochran's C test to check the assumption of homogeneity of variances (all Ps > 0.1). Prior to conducting ANCOVA, the assumption of heterogeneity of slopes was tested (all $P > 0.29$). If the result of the tests was significant, a Tukey's honestly significant difference (HSD) test was used to analyse differences between individual treatments. Data on size and swimming endurance were ln transformed to meet the assumptions of parametric analysis. All of the analyses were performed with SPSS 11.5 (SPSS Inc., Chicago, Illinois).

Results

Compensatory growth response after food deprivation

Experiment 1. Winter growth

Sticklebacks allocated to the C and RF groups were similar in size at the beginning of the experiment (MANOVA, Wilk's $\lambda = 0.88$, $F_{[2,11]} = 0.70$, $P = 0.51$). During the experiment, 13 fish of the C group (26%) and 12 of the RF group (24%) died. There was no association between food treatment and mortality rates ($\chi^2 = 0.03$, df = 1, $P = 0.853$). The mortality observed in this experiment could reflect the high natural mortality of underyearling fish during the first months of winter. (In contrast, the fish in experiment 2 experienced a very low mortality (see below) despite being kept in identical conditions, possibly because the experiment commenced after the period of high natural mortality.) Fish were measured twice during the manipulation period. By the end of the manipulation (day 35), there was a sizeable divergence between the C and RF fish (Wilk's $\lambda = 0.073$, $F_{[2,11]} = 69.37$, $P < 0.00001$) (Fig. 1), the effect of ration size being significant for both length (ANOVA, $F_{[1,12]} = 6.23$, $P = 0.0028$) and mass (ANOVA, $F_{[1,54]} = 26.04$, $P < 0.00001$). At this point, RF fish were 5.2% smaller in length and 33.5% lighter in mass than C fish.

After 8 weeks of reimposition of ad libitum rations, these differences in size between groups had become

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Fig. 1. Growth trajectories for (*a*) length and (*c*) mass of threespine sticklebacks (*Gasterosteus aculeatus*) in experiment 1 from the restricted food (RF) (open circles) and control (C) (solid circles) treatment groups; and (*b*) length and (*d*) mass of sticklebacks in experiment 2 from the RF (open circles), deprived food (DF) (triangles), and C (solid circles) groups. Arrows indicate the period of growth manipulation, after which point, all fish were on ad libitum rations. Asterisks indicate the timing of the swimming trials during the growth manipulation and compensatory period. Fish of experiment 1 were tested again 2 months after full compensation.

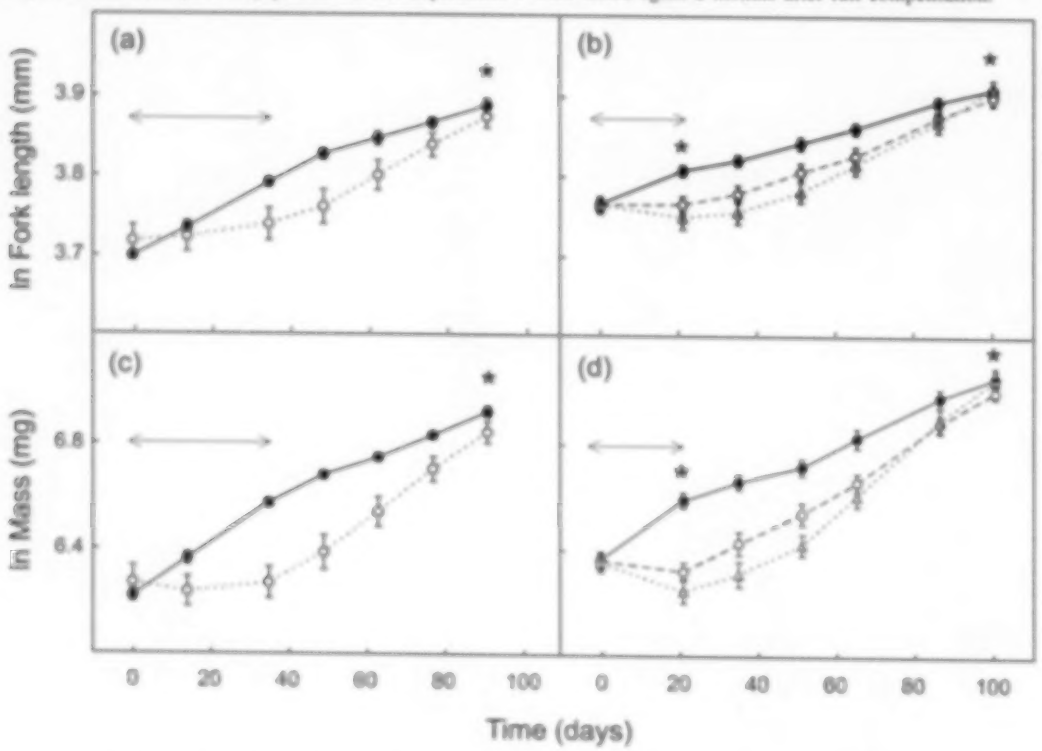

Table 1. Results of RM-ANOVA testing for effects of ration level and time (repeated measures on days 35, 49, 63, 77, and 91 since the start of the experiment, i.e., over the period following the end of the ration manipulation) on the size of threespine sticklebacks (*Gasterosteus aculeatus*) during the compensation phase of experiment 1.

(*a*) Between subjects.					
Variable	Source	SS	df	*F*	*P*
Length	Ration	0.03	1	5.388	0.039
	Error	0.066	12		
Mass	Ration	0.687	1	16.313	0.002
	Error	0.505	12		
(*b*) Within subjects.					
Variable	Source	Wilk's λ	df	*F*	*P*
Length	Time	0.031	4,9	70.144	<0.0001
	Time × ration	0.367	4,9	3.883	<0.0001
Mass	Time	0.027	4,9	82.254	<0.0001
	Time × ration	0.268	4,9	6.143	0.011

nonsignificant (Wilk's $\lambda = 0.707$, $F_{[2,11]} = 2.281$, $P = 0.15$) (Fig. 1) both in length ($F_{[1,12]} = 0.652$, $P = 0.43$) and in mass ($F_{[1,12]} = 2.926$, $P = 0.11$). The catch-up growth was confirmed by the analysis of growth trajectories. During the compensatory phase (days 35–91), the RM-ANOVA showed significant effects of ration and time on length and mass and a significant ration × time interaction (Table 1), indicating convergence of the length trajectories (Fig. 1*a*).

Experiment 2. Spring growth

Differences in size between the fish of the three treatments were nonsignificant at the beginning of the experi-

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Table 2. Results of RM-ANOVA testing for effects of ration level and time (repeated measures on days 21, 35, 51, 65, 86, and 100 since the start of the experiment (i.e., over the period following the end of the ration manipulation)) on the size of threespine sticklebacks (*Gasterosteus aculeatus*) during the compensation phase of experiment 2.

(*a*) Between subjects.					
Variable	Source	SS	df	*F*	*P*
Length	Ration	0.039	2	4.958	0.019
	Error	0.072	18		
Mass	Ration	1.076	2	11.491	0.001
	Error	0.843	18		
(*b*) Within subjects.					
Variable	Source	Wilk's λ	df	*F*	*P*
Length	Time	0.024	5,14	113.487	<0.0001
	Time × ration	0.303	10,28	2.284	0.042
Mass	Time	0.012	5,14	239.573	<0.0001
	Time × ration	0.059	10,28	8.720	<0.0001

Fig. 2. Mean ± SE swimming endurance (ln transformed) of threespine sticklebacks (*Gasterosteus aculeatus*) in experiment 1 from the control (C) and restricted food (RF) groups. (*a*) After full compensation ($F_{[1,12]} = 3.675$, $P = 0.08$); (*b*) 2 months after full compensation ($F_{[1,12]} = 0.461$, $P = 0.51$). Data are expressed as least square means (using fish length as covariate) to control for differences in body size between treatment groups at this time.

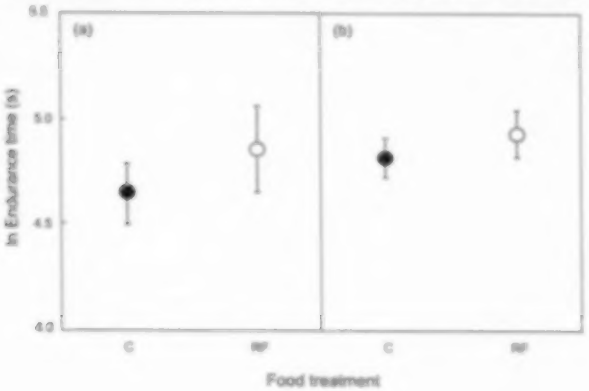
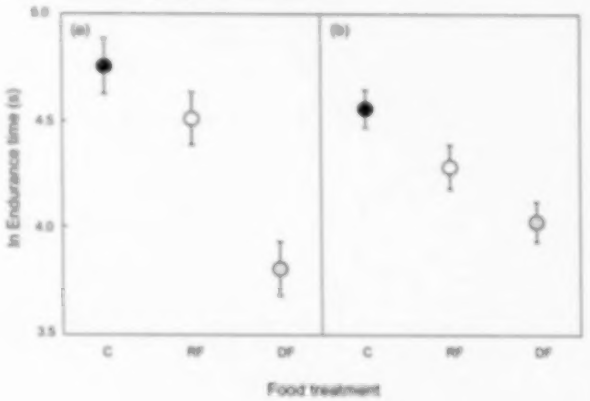


Fig. 3. Mean ± SE swimming endurance (ln transformed) of threespine sticklebacks (*Gasterosteus aculeatus*) in experiment 2 from the control (C), restricted food (RF), and deprived food (DF) groups. (*a*) After the growth manipulation period ($F_{[2,18]} = 17.434$, $P < 0.0001$); (*b*) after full compensation ($F_{[2,18]} = 8.650$, $P = 0.002$). Data are expressed as least square means (using fish length as covariate) to control for differences in body size between treatment groups at this time.



ment (Wilk's $\lambda = 0.997$, $F_{[4,34]} = 0.012$, $P = 0.99$) (Fig. 2). During the experiment, three of the C group fish, six of the RF group, and four of the DF group died. There was no association between food treatment and mortality rates ($\chi^2 = 1.15$, df = 2, $P = 0.56$). After 21 days of growth manipulation, differences in size were significant (Wilk's $\lambda = 0.148$, $F_{[4,34]} = 13.603$, $P < 0.0001$) (Fig. 1) both in length ($F_{[2,18]} = 7.258$, $P = 0.005$) and in mass ($F_{[2,18]} = 25.125$, $P < 0.0001$). At the end of the food manipulation period, RF fish were 5.3% shorter and weighed 35.5% less than C fish, while for DF fish, the values were 5.8% and 39.8%, respectively.

Seventy-nine days after the end of the manipulation period, the differences in size between groups were again nonsignificant (Wilk's $\lambda = 0.857$, $F_{[4,34]} = 0.681$, $P = 0.61$) (Fig. 1). Separate ANOVAs indicated the lack of differences in both length ($F_{[2,18]} = 0.530$, $P = 0.598$) and mass ($F_{[2,18]} = 1.401$, $P = 0.272$). The analysis of growth trajectories was consistent with these results. An RM-ANOVA showed significant effects of food level and time on length and mass and also a significant ration × time interaction (Table 2).

There was no difference between the two experiments (winter and spring growth) in growth rates of fish in the RF treatment during the compensation period at the end of the catch-up growth period (ANCOVA, $F_{[1,14]} = 0.625$, $P = 0.44$). This indicates that the RF fish had similar growth trajectories in the two experiments.

Swimming stamina

Experiment 1. Winter growth

At the end of the period of growth compensation, there was no difference in swimming endurance between the control fish and those that had experienced catch-up growth

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(nested ANCOVA, $F_{[1,12]} = 3.675$, $P = 0.08$; tank(treat): $F_{[12,27]} = 0.420$, $P = 0.94$) (Fig. 2). Two months later, the differences in swimming stamina remained nonsignificant (nested ANCOVA, $F_{[1,12]} = 0.461$, $P = 0.51$; tank(treat): $F_{[12,27]} = 1.123$, $P = 0.38$) (Fig. 2).

Experiment 2. Spring growth

As expected, the fish that had experienced a period of either food deprivation or food restriction had poorer swimming endurance than the controls after the manipulation period (nested ANCOVA, $F_{[2,18]} = 17.43$, $P < 0.0001$; tank(treat): $F_{[18,41]} = 0.802$, $P = 0.68$) (Fig. 3). When tested again at the end of the compensation period, the fish that had undergone catch-up growth still had poorer swimming endurance that those that had grown normally (nested ANCOVA, $F_{[2,18]} = 8.650$, $P = 0.002$; tank(treat): $F_{[18,41]} = 1.89$, $P = 0.046$) (Fig. 3). On average, fish from the C group were better swimmers than those that had suffered a period of food restriction (Tukey's HSD test, $P = 0.016$) or food deprivation ($P < 0.0001$). Moreover, the faster the growth rate during the catch-up period, the poorer was the subsequent swimming performance (Tukey's HSD test, C > RF > DF) (Fig. 3).

Discussion

The results of the present study support previous work that has shown the existence of compensatory growth in threespine sticklebacks after a period of food deprivation (Wu et al. 2002; Ali et al. 2003). Moreover, we demonstrated that this catch-up growth could involve significant costs in swimming performance, but these costs varied according to the time of year in which the rapid growth occurred. When the growth spurt occurred just before the breeding season, the costs in swimming performance were substantial (a reduction in swimming endurance of 13.2% in the DF fish and 6.4% in the RF fish, even when the fish had caught up fully in both weight and body length). However, there was no apparent impairment of swimming performance if the period of rapid growth occurred 2 months before the breeding season. In this earlier experiment, there was also no difference in swimming endurance of compensating and control fish when the fish were retested in May, the same month in which there were significant differences in performance in the later experiment. The difference in outcome between the two experiments is not a consequence of a more severe deficit in the second experiment, since the difference in size between C and RF fish after the period of food manipulation was similar in both experiments, and the period of food manipulation was in fact shorter in the spring experiment. This suggests that the changes in swimming endurance were due to differences in the timing of the periods of food deficit and catch-up growth rather than to the timing of the swimming trials. The explanation may lie in the resource allocation trade-off that was faced just prior to the breeding season between investment in muscular development and investment in reproduction (eggs in females and sexual ornaments in males). It is clear that reproduction has an impact on growth: in many species of fish, the allocation of energy resources to gonad production and increasing activity associated with reproduction results in growth arrest (Wootton 1982, 1999).

There is evidence that rapid growth can influence muscle cellularity and development (Galloway et al. 1999; Johnston et al. 2002), which may affect swimming ability. In some species of fish, fast-growing individuals have a higher percentage of small-diameter white muscle fibres (Weatherley et al. 1988) and greater numbers of similar-diameter red muscle fibres (Valente et al. 1999) than slow-growing individuals, which could influence fish swimming ability. Several studies have demonstrated that faster-growing fish have poorer swimming performance (Kolok and Oris 1995; Billerbeck et al. 2001), suggesting that there was a trade-off between growth rates and swimming performance. This negative relationship between growth rate and swimming performance has also been observed in larval anurans (Arendt 2003). Some authors have suggested that this trade-off is caused because a fast growth rate may limit metabolic scope and so reduce the energy available for locomotion (Conover and Schultz 1997; Billerbeck et al. 2001). The principle of allocation (Levins 1968; Sibly and Callow 1986) suggests that the allocation of energy to growth may limit the energy available for active metabolism and thus diminish locomotor capacity. Contrary to these results, in experiment 1, fish did not show a reduction in swimming performance after a period of rapid growth. This might be due to the fact that in our study, the swimming trials were performed when the fish had finished their period of rapid growth. On the other hand, the results of experiment 2 suggest that the costs in swimming performance depend on the seasonal timing of rapid growth, since in this experiment, swimming performance was reduced both during and after the period of accelerated growth. Moreover, these costs were not directly related to differences in growth rate, since the growth rates of the RF fish did not differ between the two experiments at the time of the swimming tests.

Prolonged swimming is an aerobic form of locomotion that is directly related to the prey capture ability of stream-dwelling fish (Domenici and Blake 1997). Swimming performance may also influence the capacity to perform social interactions such as aggression or sexual displays (Wootton 1976). It is therefore possible that those fish that have grown rapidly just prior to the breeding season may be less competitive and have a reduced mating success. However, a period of similarly rapid growth earlier in the year does not appear to carry a locomotory cost. This may be because of the lower requirement for investment in reproductive organs at this time and hence a greater capacity to invest in adequate muscle development.

In fish, body size is often highly correlated with breeding success. In the case of threespine sticklebacks, an increase in body length has a positive effect on reproductive success in both sexes (Kraak et al. 1999). While the exact benefits of a larger body size are likely to differ between males and females, we suggest that both sexes may be willing to incur the swimming costs of catch-up growth just prior to the breeding season to maximize their expected reproductive success.

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